THE STALING OF COFFEE

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(Received for publication, December 4, 1936)

INTRODUCTION

Broad investigations on the chemistry of coffee from many angles have been carried out by various workers and a vast literature has accumulated. The present work deals with but one phase of the study, namely, substances involved in changes which the roasted product undergoes when kept for extended periods whereby the modification of flavor known variously as "flatness," "staleness," or "rancidity" results. Since these changes are more marked in ground coffee than in the roasted whole bean, and more readily observed in coffee freshly exposed to air than in coffee not so exposed, they are commonly regarded as oxidative in character. In recent years it has been assumed in some quarters that it is oxidation or saturation of fats normally occurring in the bean which has been the principal cause of "staleness" or "rancidity." However, there are reasons for believing that the oxidation theory is inadequate to explain all changes which are brought about. Since the literature of coffee is so voluminous, reference can be made to only a few papers which have dealt with the aspect of the work with which this article is concerned.

At the present time the knowledge as to chemical changes responsible for loss or modification of flavor as exhibited in freshly roasted coffee is far from complete. We find statements such as that of Wendt (1930) who says that rancidity is due to oxidation of fat of the coffee bean, and that of Trigg (1919) claiming that deterioration is due to hydrolysis, alteration, and volatilization of its aromatic principles. More recently statements from the Continental Can Research Fellowship at the Mellon Institute (Weidlein, 1934) attribute staleness to oxidation and volatilization of substances causing aroma and to oxidation or rancidity of coffee oil. While such statements may contain considerable truth, no experimental data are given to support them, and they are too general to provide a satisfactory explanation.

A systematic investigation of the chemistry of deterioration of coffee has been carried on for many months by Bengis and Anderson (1932, 1934) and they have reported isolation of a constituent of the unsaponifiable matter which they term "Kahweol." This is an

This investigation was made possible through a generous contribution from the American Can Company.

unsaturated substance which changes very rapidly in presence of air or acids. They have also attempted to follow changes taking place in coffee fat during a period of sixteen months, but could detect only very slight changes in the amount of extractible fat, unsaponifiable content, iodine value, and saponification value of the fat or in the ratios of the various acid components of these glycerides during this period.

Several years ago some preliminary studies on rancidity of coffee were undertaken by the authors, results of which appeared in a "Report on an Investigation of Coffee" published by the Joint Coffee Trade Publicity Committee. After a considerable hiatus during which it was our intention to resume the investigation, and before the appearance of the papers of Bengis and Anderson (1932, 1934) on changes in coffee fat, investigations were resumed in this laboratory. At that time it seemed likely that fat played an important role in staleness of coffee, and this was the first aspect of the problem to be considered.

EXPERIMENTAL WORK

During the earlier work carefully prepared samples of coffee extracts had been made. Instead of waiting for coffee to age, therefore, these samples, which were undoubtedly in a more advanced state of deterioration than those examined by Bengis and Anderson (1932, 1934), were subjected to certain chemical tests. The samples gave clear evidence of deteriorative change and were investigated with reference to acid value, saponification number, percentage of unsaponifiable matter, iodine value, and peroxide content. These were compared with fresh coffee extracts prepared in a similar manner, with extracts five and one-half months old, and also with freshly prepared extract of ground coffee which had been kept in ground condition for five and one-half months. The comparative results of these examinations are summarized (Table 1).

In order to place on record the methods followed in this investigation the following descriptions of the extracts are presented:

Description of Extracts

Extract *A* was obtained by continuous extraction of freshly roasted and ground coffee with petroleum ether which had been freed from unsaturated hydrocarbon by refluxing over concentrated sulfuric acid, washing with water, and distilling over stick sodium hydroxide. Only that portion of the petroleum ether which boiled from 42 to 80°C. (107.6 to 176°F.) was used. After extraction of the fat, the petroleum ether was removed by distilling on a water bath. The final stages of distillation were carried out under reduced pressure. The temperature of the water bath never exceeded 70°C. (158°F.). The weight of the extract was 8.2 per cent of that of the coffee used. It was a dark brown liquid of fairly low viscosity and surface tension and had a pleasant coffee odor.

Extract *B* was a five and one-half months old petroleum ether extract of Santos coffee. It had remained on a laboratory bench protected from dust and unprotected from light or air during that time. It was a dark brown liquid of fairly low viscosity and had a slightly rancid coffee odor.

Extract *C* was a fresh extract of the same lot of coffee from which extract *B* was prepared; it had been aged in the ground condition for five and one-half months. The extract was prepared in the same manner as *A*. It weighed eight per cent as much as the ground coffee used.

Extracts *D*, *E*, *F*, and *G* were similarly prepared extracts which had aged a much longer time. Their exact age is not a matter of record. They no longer

TABLE 1

Changes in the Chemical Characteristics of Coffee Fat Owing to Age

	Fresh coffee	An extract 5½ mo. old	Fresh extract of coffee 5½ mo. old	Extracts much older			
	A	*B*	*C*	*D*	*E*	*F*	*G*
Acid value in milligrams of KOH/gm...	3.3	4.9	5.2	18.7	29.9	50.4	47.2
Saponification No. in milligrams of KOH/gm.....	171	174	172	197	192	206	200
Unsaponifiable per cent.....	9.5	7.5	.....	.....	5.0	4.2	.....
Iodine value (Hubl) in centigrams of iodine/gm.....	90.9	84.7	90.7	54.2	49.1	34.3	.....
Peroxide content in millimoles/kg.....	0	80.5	.....	199	138	49	.....

had the odor of coffee. They were chosen in the order of increasing deterioration as well as could be judged by their appearance and odor. A brief description of each is as follows:

D. A light-colored extract; solvent used unrecorded; consistency about the same as heavy-bodied linseed oil; no crystals in the liquid.

E. A dark acetone extract; more viscous than *D*; containing some gummy material settling on the bottom of the flask.

F. A fairly light-colored petroleum ether extract; more viscous than *A* or *B*, but still possible to pour; contained a large amount of crystalline material.

G. A dark, gummy, semisolid extract; solvent unrecorded; contained a large amount of crystalline material.

The results show clearly that the fat in coffee is susceptible to oxidation, but that this change has been only partial in five and

one-half months. This fact is quite contrary to the opinion of at least one writer, Bredt (1934), who claims that while extracted fats of coffee grow rancid rather slowly in the mass and under ordinary conditions, the alteration occurs rapidly when they remain in contact with the great surface actually exposed in a large quantity of coffee beans.

In opposition to this view our data on the lower iodine value and the high peroxide value of the fat kept five and one-half months after extraction would indicate that decomposition took place more rapidly when the fat is in compact form than when it is dispersed. This difference is probably due to action of light in one case and inaccessibility of fat in the bean to atmospheric oxygen in the other.

During an aging period of five and one-half months only the acid and peroxide values changed sufficiently to give any promise as a means of measurement of staleness or rancidity. Peroxide contents have been used by Greenbank and Holm (1934), Wheeler (1932), Lea (1931), and others to detect rancidity in fats and oils. Although Coe and LeClerc (1934) have recently cast some doubts upon the value of such determinations as a measure of rancidity, peroxide content is still considered the most reliable test for rancidity of fats. For this reason it seemed desirable to measure the peroxide contents of the same coffee at different degrees of staleness to see if any relation existed between this constant and the degree of deterioration. After considerable experimentation with various solvents and methods of extraction the following procedure was found quite satisfactory.

Ten grams of ground coffee were placed in a ground glass-stoppered Erlenmeyer flask with 50 c.c. of carbon tetrachloride which had previously been shaken with a little mercury to remove traces of chlorine. The mixture was shaken in a shaking machine for three hours. At the end of that time 25 c.c. of the solvent were filtered off and placed in another ground glass-stoppered flask; 1 c.c. of a saturated solution of potassium iodide in water and 25 c.c. of glacial acetic acid were added. After the mixture had stood for 20 minutes in the dark, 100 c.c. of water and a little starch indicator solution were added. The mixture was then titrated with 0.002 N sodium thiosulfate until the water layer became colorless. The "peroxide content" in millimoles of peroxide per kilogram of coffee was computed as follows:

$$2 \times \frac{\text{c.c. of 0.002 N thiosulfate}}{10} = \text{"peroxide content"}$$

The value may be somewhat in error, as the 25 c.c. of solvent filtered off probably does not contain exactly one-half of the peroxides

in the 10 grams of coffee. However, the error is probably fairly constant and the values obtained comparable.

The time of decomposition of the peroxides before titration was varied with the following results:

Length of time	Peroxide value
15 min.	0.47
20 min.	0.47
25 min.	0.51
30 min.	0.54

The higher values obtained on 25 and 30 minutes' standing are probably due to oxidation of the hydrogen iodide by atmospheric oxygen. The differences are slight, and 20 minutes was chosen as the best time.

The amount of iodine adsorbed by the coffee fat was estimated by extracting a sample of freshly roasted coffee containing no peroxides, exactly as in the determination above, and then adding a known

TABLE 2

Effect of Aging on Coffee

Description of coffee	Peroxide content	Acid value	Taste test
Coffee 7 mo. old kept in the bean, freshly ground just previous to testing	0	6.4	Tasteless but not rancid
Same as above, but kept 7 mo. in the ground state	0.50[1] 0.65[1]	5.5	Slightly rancid
Coffee 2½ mo. old, kept in the bean and freshly ground before testing	0	3.4	Stale but not rancid
Same as above, but kept 2½ mo. in the ground state	Trace	4.0	Rancid
Coffee 1 mo. old, kept in the ground state	0	4.6	Stale but not rancid

[1] Different parts of the same bag.

amount of iodine (equivalent to 7.65 c.c. of 0.002 N thiosulfate), the acetic acid, and saturated potassium iodide solution; allowing it to remain 20 minutes and completing the analysis as usual. An amount of iodine equivalent to 0.07 c.c. of 0.002 normal thiosulfate was adsorbed. This would lower the peroxide value by only 0.014 which is outside the limit of accuracy of the determination.

That neither peroxide content nor acid value is a reliable measure of the staleness of coffee is quite evident from the summary of results of the application of this determination to various samples of coffee (Table 2). One cause of the low peroxide values of some stale coffees

might be the decomposition of the peroxides to give products of unpleasant taste and smell. It seemed advisable, therefore, to study the effect of moisture on the taste, odor, peroxide content, and acid value of the coffee.

EFFECT OF MOISTURE ON COFFEE

The effect of moisture during the storing of coffee was determined by keeping one lot of coffee in a very dry atmosphere and another batch in contact with air with a relative humidity of 75 per cent. The acid values, moisture, and peroxide values were determined at intervals. Taste tests were run at the same time. The humidity was controlled by placing small crystallizing dishes containing the ground coffee in desiccators, one of which contained anhydrous calcium chloride, and the other a saturated sodium chloride solution. The salt solution gave a relative humidity of approximately 75 per cent over the range of temperatures existing in the laboratory. The dishes

TABLE 3

Effect of Moisture on Roasted Coffee

Sample kept over anhydrous calcium chloride			Weeks in storage	Sample kept over a saturated sodium chloride solution		
Acid value	Peroxide content	Moisture		Acid value	Peroxide content	Moisture
		pct.				*pct.*
3.8	0	2.38	0	3.8	0	2.38
3.9	0	.....	3	4.2	0	.....
3.9	Trace	.....	6	4.3	0	.....
4.0	0	1.4	9	4.2	0	9.1

were placed so that free circulation of air within the desiccators was possible. The coffee was frequently stirred and positions of the dishes changed so as to obtain uniform aging. The desiccators were placed in a dark closet to eliminate any effect of light.

The first test was made after three weeks. It disclosed that the coffee kept in a moist atmosphere was absolutely lacking in coffee flavor. The sample kept in a dry atmosphere was but little weaker than fresh coffee and could not be classed as stale even after six weeks had elapsed. The moisture contents, peroxide values, and acid values are shown (Table 3). The acid value is that of the extracted fat and does not depend on the moisture content of the coffee.

While this experiment shows the importance of moisture in the deterioration of coffee, it does not substantiate the hypothesis that peroxides are intermediates in this degradation, for no peroxides could be detected in the sample kept in the moisture-free atmosphere. In all this work no convincing evidence has been obtained to show that fat in coffee becomes rancid in the relatively short time that

it takes coffee to become stale. For this reason it seemed wise to turn attention to other constituents of coffee, especially to the aromatic oils which give it taste and odor. Another indication that such an investigation might be advisable was that the salt solution in the desiccator used in the experiment just described contained small amounts of furfuraldehyde; and the volatile oils of coffee have been found to contain furan derivatives.

STUDY OF VOLATILE CONSTITUENTS OF COFFEE

A number of investigators have analyzed the volatile oils from coffee. There is considerable divergence in their results. A part of this is probably due to the use of different types of coffee. Other differences may be due to variation in method of isolation of the oils. Still others must be attributed to misinterpretation of experimental results.

Summary of Previous Work

The first systematic study of the aromatic oils of coffee was undertaken by Oscar Bernheimer (1880). He obtained oils by condensing coffee-roaster gases. From these he isolated a material which he believed to be a methyl derivative of saligenin. The material which he obtained distilled from 195 to 198°C. (383 to 388.4°F.). It was the principal constituent of the liquid portion of his product. The other components found which might affect aroma were acetic acid, methylamine, pyrrol, and acetone.

If the material obtained by Bernheimer was a methyl derivative of saligenin it would have had one of the following formulas:

(I) $C_6H_4\begin{cases} OCH_3 \\ CH_2OH \end{cases}$ o-methoxybenzyl alcohol

(II) $C_6H_4\begin{cases} OH \\ CH_2OCH_3 \end{cases}$ o-hydroxybenzylmethyl ether

The first of these compounds had been prepared by Cannizzarro and Körner (1872). Bötsch (1880) pointed out that this could not be the same compound that Bernheimer had, for its physical properties were quite different. There is, for example, a difference of over 50 degrees in boiling points. He believed that it was probably the compound represented by Formula II. This was shown not to be the case, however, by Thiele and Dimroth (1889) who synthesized it. This compound certainly could not be that isolated by Bernheimer, as it could not be distilled at atmospheric pressure without almost complete resinification, while that obtained from coffee distilled at almost 200°C. (392°F.) under normal pressure. Neither of the two ethers of saligenin which were prepared synthetically resembled coffee in

odor, although Bötsch thought that his crude methoxybenzyl alcohol had coffee-like odor which disappeared on purification.

Although this work would seem to prove rather definitely that Bernheimer could not have had a methyl ether of saligenin, a number of years later Sethness (1924) published a paper in which he speaks of methyl ether of saligenin being the chief constituent of the aromatic oils of coffee. He steam-distilled coffee on a large scale and obtained a product of like boiling point. Apparently he ran no chemical tests on the material. Sethness states that Lehmann and Wilhelm steam-distilled coffee and also found caffeol, "or chemically speaking, the methyl ether of saligenin to be the principal ingredient of the fraction possessing the powerful coffee aroma." The only paper that we have been able to find by Lehmann and Wilhelm (1898) contained no statement or experimental evidence as to the chemical nature of steam distillate of coffee. Bernheimer seems to have been the only investigator to present chemical evidence that the material boiling at 195 to 198°C. (383 to 388.4°F.) was a derivative of saligenin. That it was a methyl ether of this compound seems impossible. It seems probable that Sethness and Bernheimer both obtained the same material, yet no other investigator has obtained any material resembling this product as the main constituent of the volatile oils of coffee.

Other investigators have obtained quite different results. A few years after Bernheimer, Jaeckle (1898) also analyzed the condensate from coffee-roaster gases. He identified acetone, furfuraldehyde, formic and acetic acids, pyridine, ammonia, and trimethylamine. This paper was soon followed by a report of the excellent work by Erdmann (1902). Erdmann steam-distilled large amounts of coffee and analyzed the steam distillate. He used a total of 225 kilograms of coffee in his experiments. He was the first to use the coffee itself as a starting material rather than the condensate from the roaster gases. Erdmann found about 42 per cent of his product to be acidic. This was mostly valeric acid but contained some acetic acid. The neutral portion (58 per cent) was largely furfuryl alcohol but also contained some phenols and an extremely small amount of a compound boiling at 93°C. (199.4°F.) under 12 mm. pressure. This fraction contained nitrogen.

Some years later Bertrand and Weisweiller (1913) analyzed a similar fraction and reported that the nitrogen content is due to the presence of pyridine. Grafe (1912) also analyzed the oils in coffee which were volatile with steam. He also reports valeric and acetic acids, furfuryl alcohol, phenols, furfuran derivatives, and a pyridine derivative which gives coffee its aroma. Grafe was chiefly interested in a comparison of normal and decaffeinated coffees and his work is

on a comparatively small scale. He depended largely on color reactions and odors for his identification.

More recently Schmalfuss and Barthmeyer (1929) have shown the presence of diacetyl in coffee.

A chronological tabulation of the findings from previous work on the volatile oils of coffee is included here (Table 4).

TABLE 4

Findings from Previous Work on Volatile Oils of Coffee

Investigator	Compounds identified
O. Bernheimer[1] (1880)	A methyl derivative of saligenin, caffeine, higher fatty acids, acetic acid, hydroquinone, methylamine, pyrrol, acetone.
A. Monari and L. Scoccianti[1] (1895)	Pyridine.
H. Jaeckle[1] (1898)	Furfural, caffeine, pyridine, ammonia, trimethylamine, acetic and formic acids, and acetone.
E. Erdmann (1902)	A valeric acid in large amounts, a little acetic acid, phenols, furfuryl alcohol, a fraction boiling 93° C. (199.4° F.) at 13 mm., containing nitrogen and having the odor of coffee. This last material in extremely small amounts.
V. Grafe (1912)	Acetic and valeric acids, furfuryl alcohol, furfuran compounds, phenols, and a pyridine derivative.
G. Bertrand and G. Weisweiller (1913)	Pyridine.
R. E. Sethness (1924)	Methyl ether of saligenin claimed to be the principal constituent of coffee oils.
H. Schmalfuss and H. Barthmeyer (1929)	Diacetyl.

[1] Analyzed condensate from coffee-roaster gases.

INVESTIGATION OF VOLATILE OILS IN COFFEE

a. *Extraction of Coffee by Methanol*

The problem of separation of volatile constituents from the remainder of the roasted coffee is almost as difficult and important as identification of these compounds. The percentage of volatile oil in roasted coffee is always very small, e.g., Erdmann obtained 83.3 grams of volatile oils from 150 kilograms of roasted coffee. This corresponds to about 0.05 per cent. Three methods of isolation have been used:

(1) condensation of liquids and solids in the roaster gases, (2) steam distillation of roasted coffee, and (3) extraction of roasted coffee with ether. The first of these methods does not necessarily give the same products as occur in the roasted coffee. The use of steam distillation is more reliable, but alteration of oils may occur owing to the action of the water. As has been previously shown coffee aroma is greatly affected by action of water vapor. The last method, extraction by a solvent, seemed the most promising and was tried in this laboratory, but it also had some disadvantages.

A series of preliminary tests indicated that methyl alcohol gave an extract having a very pleasant and distinct coffee odor, and this alcohol was used as a solvent. It soon became evident that extraction with this solvent gave an extract which upon distillation of the solvent left a residue which was too viscous to allow the removal of any volatile components by vacuum distillation. It was found possible to precipitate a part of this viscous material by addition of ethyl ether. Taking advantage of this fact the procedure indicated (Table 5) was developed. It was necessary to carry out the last precipitation with petroleum ether as ethyl ether was no longer effective. From the yields of volatile oils at the end of this procedure it is quite evident that some of the desired volatile components were occluded in the precipitates thrown down by the ether. The ether precipitate was semicrystalline but very difficult to purify. All attempts to filter it failed. Decantation of the supernatant liquid was resorted to. The volatile oil was shown to consist of furfuryl alcohol with a little furfuraldehyde, acetic acid, and some water-insoluble liquid which may have been an ester or esters of furfuryl alcohol. This method was finally abandoned because of the length of procedure and small yields of the desired product.

A variation of the above scheme was to add basic lead acetate to the methanol extract. Much of the viscous material in the extract will precipitate with basic lead acetate and may be separated in this manner. However, much of the volatile constituents must also be occluded in some manner, for the yields of these compounds were practically nil. Nor did this method serve as a method of purification of the non-volatile materials precipitated by the lead acetate, for upon regeneration by hydrogen sulfide they were more tarry than before.

A description of the four volatile fractions is given below.

Description of Volatile Matter Extracted from Coffee by Methanol

Fraction boiling from 77°C. (170.6°F.) at 40 mm. pressure. About 0.15 c.c. Partly miscible with water. Refractive index 1.4480 at 25.5°C. (77.2°F.).

TABLE 5

Extraction of Roasted Coffee with Methanol

13 kilograms coffee + 21 liters methanol

Centrifuge

Coffee grounds (discarded)	Methanol extract
	Distil off CH_3OH in an atm. of CO_2
	Thick concentrated extract
	Add ether
Tarry material (attempts at purification futile)	Ether + methanol solution
	Concentrate in an atm. of CO_2
	Thick concentrate
	Add ether
Tarry material	Ether + methanol solution
Solid, m.p. 232-236° C. on an immersion thermometer (probably caffeine)	Concentrate in an atm. of CO_2
	Thick concentrate
	Add ether
	Solution (*no* separation)
	Add petroleum ether
Tarry material	Petroleum ether + ether + methanol
	Solution
	Concentrate
	Concentrated extract
	Vacuum distil { (Mineral oil added to reduce frothing)
Residue (discarded)	Four fractions

Gave a strong fuchsin aldehyde test. Vapors colored aniline acetate paper red. Formed a dimethon whose crystalline structure resembled that from furfuraldehyde when viewed under a microscope. It was mixed with small droplets of oil.

From the above data it was concluded that this fraction was largely furfuraldehyde. The boiling point of furfuraldehyde is 77 to 78°C. (170.6 to 172.4°F.) at 40 mm. pressure.

Fraction boiling from 88 to 94°C. (190.4 to 201.2°F.) at 40 mm. pressure. About 0.3 c.c.

About one-half soluble in water. A small amount treated with a naphthylisocynate gave a urethane melting at 127 to 128°C. (260.6 to 262.4°F.). The urethane from furfuryl alcohol melts at 129°C.

After extracting with water this fraction had a refractive index of 1.4628 at 25.5°C. (77.2°F.). The refractive index of furfuryl formate is 1.4672; that of furfuryl acetate, 1.4604; both at 25.5°C. (77.2°F.). This may be one or a mixture of these esters.

Fraction boiling 92 to 100°C. (197.6 to 212°F.) at 4.5 mm. pressure. About 0.1 c.c.

Saponification value 47.

Fraction boiling at 109°C. (228.2°F.) at atmospheric pressure. About 1 drop.

Identified as acetic acid by the p-nitrobenzyl ester, m.p. 78°C. (172.4°F.).

b. *Extraction of the Coffee Brew*

From experience with extracting coffee with methanol we felt quite certain some method of separation of volatile constituents from other extractible material must be devised. A change in solvent was also considered, but even with a solvent like petroleum ether, which extracts a comparatively small amount of material, the ratio of non-volatile to volatile constituents in the extract is very high. One simple method of separating the desired volatile oils from other materials extracted by organic solvents is to make first a coffee brew (water extract) and then extract it with an organic solvent. An extraction apparatus was devised in which the coffee brew was dropped in a fine spray through columns of organic solvent five feet long. At first three solvents were used in succession, ethyl ether, chloroform, and ethyl acetate. Chloroform was then eliminated because it extracted the same compounds as ethyl ether and necessitated a bothersome type of apparatus in which the brew passed up through the solvent rather than being dropped down through it as in the case of the lighter solvents.

A test was also run to determine the number of five-foot tubes of ether necessary for complete extraction of the coffee. Practically nothing could be extracted after the brew had passed through 27 tubes and the amount of volatile material obtained from the nineteenth to twenty-seventh tube was hardly sufficient to warrant the in-

crease in time necessary for their use. The nature of the extracted oil seemed the same as in the previous tubes.

The scheme followed consists of making 10.5 kilograms of Santos coffee into 45 liters of brew. The coffee is roasted in the laboratory within a few hours of the time it is used. A medium roast is employed. The coffee brew is then passed through 20 tubes of ethyl ether, five feet long and about two centimeters in diameter. After passing through the ether, the coffee is then dropped down 10 tubes of ethyl acetate. Impurities in the ethyl acetate interfered with identification of any compounds extracted from the coffee brew, so it is uncertain at present if this extraction is worth while.

The ether in the tubes was changed after nine liters of brew had passed through. The ether extract was dried over anhydrous sodium sulfate and then anhydrous calcium sulfate (Drierite). The extract was concentrated by distilling off the ether in an atmosphere of carbon dioxide gas. Usually the concentration was interrupted when the volume of the solution had reached about 200 c.c. and this was then combined with other similar batches. After again drying over anhydrous calcium sulfate the greater part of the ether was removed from the combined extracts by distillation on a water bath. Carbon dioxide gas was passed through the apparatus to reduce the amount of oxidation. The final stages of the distillation were carried out under reduced pressure. The receiver was covered with dry ice so as to condense all the distillate. The pressure was reduced as low as possible so as to reduce the temperature of distillation. Usually two to four millimeters were obtained. No attempt was made to determine the boiling points, as superheating always occurred. The distillate was carefully redistilled fractionally later. The temperature of the oil bath used for heating was never over 160°C. (320°F.).

c. *Analysis of Ether Extract of Coffee Brew*

The distillate from the ether extract was separated into the following fractions:

A. That distilling at room temperature under 5 mm. pressure. About 1.2 c.c.

B. That distilling up to 70°C. (158°F.) at 40 mm. About 2 c.c.

C. That distilling from 70 to 100°C. (158 to 212°F.) at 40 mm. About 4 c.c.

D. That distilling from 100°C. (212°F.) at 40 mm. to 120°C. (248°F.) at 3 to 4 mm. About 1.5 c.c.

These fractions were treated as follows:

Fraction A.

Redistilled, this fraction boiled from 70 to 78°C. (158 to 172.4°F.) at atmospheric pressure. It was almost completely water soluble. It was identified as ethyl alcohol by the a-naphthylcarbamate, m.p. 77 to 78°C. (170.6 to 172.4°F.).

Fraction B.

On redistillation nearly all of this fraction boiled at 110 to 118°C. (230 to 244.4°F.). A little resinified material was left in the bottom of the distilling flask. It had an acid value of 124. Acetic acid has a theoretical value at 167. It was identified as acetic acid by the p-bromophenacyl ester, m.p. 85 to 86°C. (185 to 186.8°F.). The acetic ester of p-bromophenacyl alcohol melts at 86°C. (186.8°F.). A mixed melting point with the derivative prepared from acetic showed no depression. This fraction is evidently essentially acetic acid.

Fraction C.

An acid value showed that this fraction still contained considerable acid. The fraction gave about 0.5 c.c. of liquid distilling below 70°C. (158°F.) at 40 mm. The remainder distilled from 77 to 100°C. (170.6 to 212°F.) under 40 mm. pressure. The last fraction was largely soluble in water. After shaking with several times its volume all but 0.5 c.c. dissolved. The water-insoluble portion was dried over anhydrous sodium sulfate and then over anhydrous calcium sulfate and distilled. A few drops distilled at 77 to 85°C. (170.6 to 212°F.) at 40 mm. This gave a strong aniline acetate reaction. This is probably largely furfuraldehyde.

The remainder of the water-insoluble portion distilled at 95 to 98°C. (203 to 208.4°F.) at 40 mm. A saponification value corresponded to about 50 per cent ester calculated as furfuryl acetate. B.P. of furfuryl acetate 95 to 97°C. (203 to 206.6°F.) at 40 mm. The water solution of the soluble components was saturated with salt and extracted several times with ether. These ether extracts were combined and dried over anhydrous calcium sulfate.

After the ether was removed by distillation the residue distilled at 91 to 94°C. (195.8 to 201.2°F.) at 40 mm. Furfuryl alcohol boils at 92 to 94°C. (197.6 to 201.2°F.) at 40 mm. This material was identified as furfuryl alcohol by its a-naphthylcarbamate, m.p. 127 to 128°C. (260.6 to 262.4°F.). The melting point of the a-naphthylcarbamate from furfuryl alcohol is given as 129°C. (264.2°F.). A mixed m.p. with the urethane prepared from furfuryl showed no depression. The residue in the flask (1 to 2 drops), after the distillation of the material shown to be furfuryl alcohol, smelled like phenol or the cresols. The flask was washed out with water and a drop of very dilute ferric chloride solution added. A deep violet color appeared. To another portion of the water solution bromine water was added. A white turbidity appeared. However, practically no precipitate settled out on standing.

The one-half cubic centimeter of material distilling below 70°C. (158°F.) at 40 mm. was redistilled. It distilled at 115 to 140°C. (239 to 284°F.) at atmospheric pressure. The greater part of it distilled from 115 to 120°C. (239 to 248°F.). There was a little tarry material left in the flask. The distillable material was identified as acetic acid by making the p-bromophenacyl ester, m.p. 85 to 86°C. (185 to 186.8°F.).

From the above we can conclude that this fraction was one-eighth acetic acid. The remainder was nearly all furfuryl alcohol but also contained some ester, probably furfuryl formate, and a small amount of a phenol.

Fraction D.

The material in this fraction was very viscous; it was redistilled. Only about 1 c.c. distilled. Most of this boiled at 95 to 103°C. (203 to 217.4°F.) at 1 to 1.5 mm. There was considerable loss in manipulation owing to the high viscosity of the material. A sodium fusion followed by a cyanide test showed the presence of

nitrogen. It had an acid value of 34.5 (c.c. 0.1 normal alkali per gram). It was shaken with several times its volume of water. The insoluble oil was separated and dried over anhydrous sodium sulfate and then over anhydrous calcium sulfate. This water-insoluble portion still gave a positive but much weaker nitrogen test. It had an acid value of 34.7. Phenolphthalein was used as the indicator and the end point was sharp. The material was very viscous. It gave a light violet color with dilute ferric chloride solution. There was too little left to test further.

The water solution of the water-soluble components was saturated with salt and extracted several times with ethyl ether. These extracts were combined and dried over sodium sulfate and then over calcium sulfate. The ether was removed by distillation. The residue was quite fluid. It had an acid value of 33.5. The end point was poor and the red color of phenolphthalein kept increasing as more alkali was added. This residue contained nitrogen. The test was much stronger than in the case of the water-soluble portion. The color formed by the addition of a few drops of dilute ferric chloride solution to a water solution of the material was a very intense violet. This was much stronger than in the case of the water-insoluble portion. A water solution reduced Tollen's reagent immediately at room temperature. It did not give Feigl's (1934) test for primary aromatic amines with 4-pyridyl pyridium chloride solution. Apparently it is not positive that the substance is not a primary aromatic amine, as methyl anthranilate did not give the test, although anthranilic acid gave a very fine positive result.

From the above data it seems likely that the viscous, water-insoluble material is an acid. It may be a fatty acid from the coffee fat. Its acid value is about right. The water-soluble portion is probably a phenol. The indistinct end point color with ferric chloride and reduction of Tollen's reagent all point to this.

Redistillation of Last Portions of Ether Which Distilled at Atmospheric Pressure

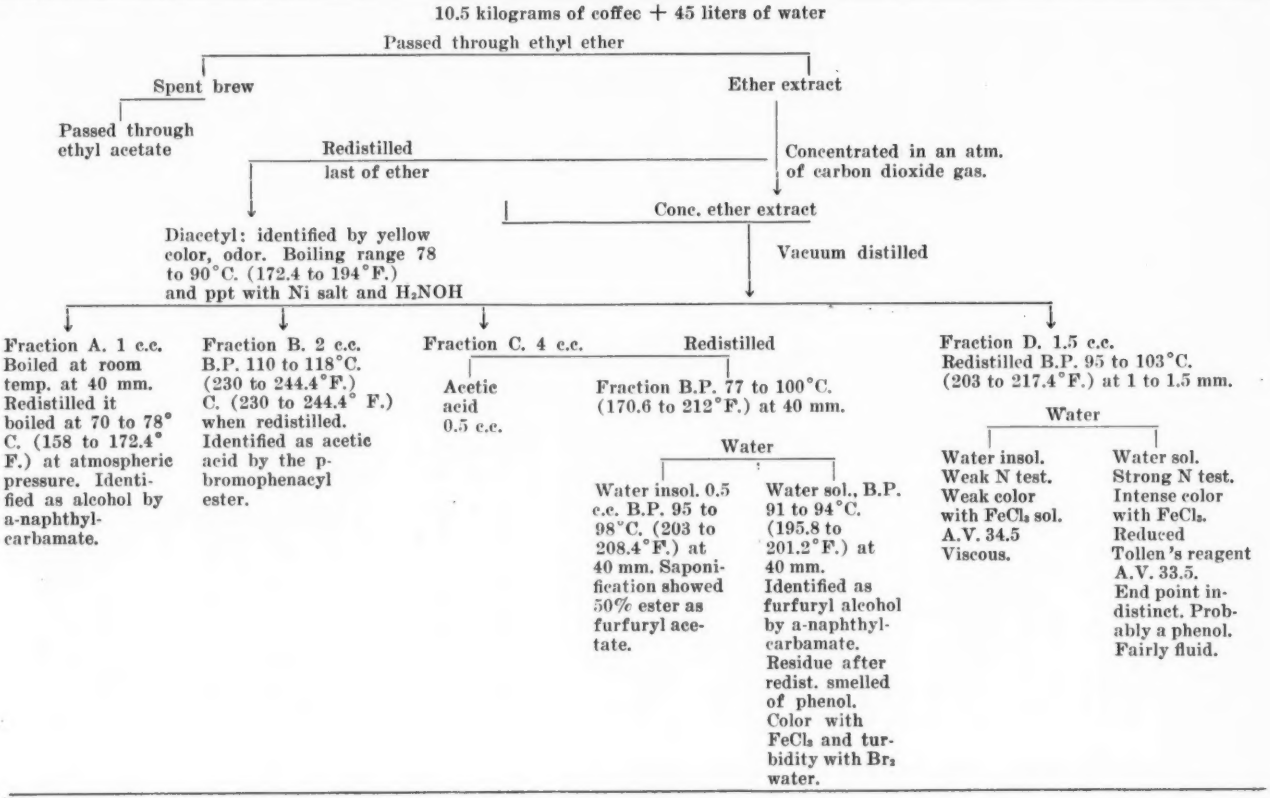
The last few cubic centimeters of ether to distill at atmospheric pressure had been saved from several runs. These were combined and distilled through a good column having a water reflux. After a small quantity distilled, the distillate, between 78 and 90° C. (172.4 and 194° F.), became very yellow and smelled of diacetyl. A little of the distillate was extracted with water and treated with nickel sulfate, hydroxylamine sulfate, and ammonia. The bright red nickel dimethylglyoxime precipitated. The fraction was nearly all soluble in water and smelled of alcohol. The extraction of the coffee by ether and the identification of the extracted compounds is summarized in tabular form (Table 6).

DISCUSSION OF PRESENT WORK AND THAT OF PREVIOUS INVESTIGATORS

The results of the present investigation do not wholly agree with those of any previous investigation. We agree with Grafe (1912) and Erdmann (1902) that furfuryl alcohol is the chief neutral constituent

TABLE 6

Extraction of Coffee Brew with Ether


10.5 kilograms of coffee + 45 liters of water
Passed through ethyl ether
Spent brew
Ether extract
Passed through ethyl acetate
Redistilled last of ether
Concentrated in an atm. of carbon dioxide gas.
Conc. ether extract
Diacetyl: identified by yellow color, odor. Boiling range 78 to 90°C. (172.4 to 194°F.) and ppt with Ni salt and H2NOH
Vacuum distilled
Fraction A. 1 c.c. Boiled at room temp. at 40 mm. Redistilled it boiled at 70 to 78° C. (158 to 172.4° F.) at atmospheric pressure. Identified as alcohol by a-naphthyl-carbamate.
Fraction B. 2 c.c. B.P. 110 to 118°C. (230 to 244.4°F.) C. (230 to 244.4° F.) when redistilled. Identified as acetic acid by the p-bromophenacyl ester.
Fraction C. 4 c.c.
Redistilled
Acetic acid 0.5 c.c.
Fraction B.P. 77 to 100°C. (170.6 to 212°F.) at 40 mm.
Water
Water insol. 0.5 c.c. B.P. 95 to 98°C. (203 to 208.4°F.) at 40 mm. Saponification showed 50% ester as furfuryl acetate.
Water sol., B.P. 91 to 94°C. (195.8 to 201.2°F.) at 40 mm. Identified as furfuryl alcohol by a-naphthyl-carbamate. Residue after redist. smelled of phenol. Color with FeCl3 and turbidity with Br2 water.
Fraction D. 1.5 c.c. Redistilled B.P. 95 to 103°C. (203 to 217.4°F.) at 1 to 1.5 mm.
Water
Water insol. Weak N test. Weak color with FeCl3 sol. A.V. 34.5 Viscous.
Water sol. Strong N test. Intense color with FeCl3. Reduced Tollen's reagent A.V. 33.5. End point indistinct. Probably a phenol. Fairly fluid.

of aromatic oils of coffee. Erdmann found a valeric acid, however, to be the main acid component. Acetic was found to be the principal acid in the present work. There can be little doubt that this was the acid occurring in our products, as both the odor and boiling points of the two acids could not be mistaken. In addition to this we have the additional evidence of a mixed melting of the p bromophenacyl ester of the acid from the coffee and that from acetic acid. Santos coffee was used in both researches.

The method of isolation of the oils was different and may possibly have something to do with this divergency. Grafe reports that his acid fraction smelled of acetic acid but calculates it as a valeric acid on titration. He gives no derivatives. Both Erdmann and Grafe agree that coffee owes its odor to a nitrogen-containing compound. Sethness (1924) also reports nitrogen in his "caffeol" fractions. The high-boiling, water-soluble fraction which we found had a fine coffee aroma. It contained nitrogen and may be the compound to which coffee owes its delicate aroma. However, we have found that it is very dangerous to judge by the odor of materials extracted. Extracts having a good coffee aroma may be almost entirely composed of materials of different aromas or of practically no aroma. Whatever compound gives coffee its pleasant odor must occur in extremely small amounts and be capable of scenting huge amounts of material.

As pointed out in the discussion of the previous work, it has been rather well established that Bernheimer (1880) could not have had a methyl derivative of saligenin. However, we examined our extracts for indications of the presence of this compound. It certainly was not a major constituent of our volatile oils. Both ethers of saligenin boil 20 to 40 degrees higher than the materials obtained by us when distilled at 40 mm. It is hard to believe that a difference in the type of coffee used would make this difference, for Grafe gave evidence that the furfuryl alcohol probably comes from decomposition of the hemicellulose of the thick endosperm cells. It seems that this reaction should occur regardless of the type of coffee used. Of course the amount would vary. It is really difficult to see why Bernheimer and Sethness did not obtain any furfuryl alcohol. The boiling points of furfuryl alcohol and the caffeol reported by Bernheimer are over 20 degrees apart, and Bernheimer also obtained salicylic acid from his material. Sethness checked his boiling point but gave no derivative.

RELATION OF STALENESS TO CHEMICAL COMPOSITION OF VOLATILE OILS IN COFFEE

The mixture of volatile materials extracted from coffee is very unstable. This instability is probably due to the peculiar composition

of the mixture. It soon became apparent in this work that furfuryl alcohol is unstable in the presence of even very small amounts of acid. If the mixture of volatile ingredients freshly extracted from the coffee be kept for a short time, much darkening and finally resinification takes place. If exposed to air this change is very evident even overnight. Sealed in an evacuated tube the change is much retarded. After the acid is separated from the mixture by distillation, however, a much greater stability is noticed in the furfuryl alcohol fraction.

In order to test whether this decomposition could have anything to do with the unpleasant taste of stale coffee small portions of a freshly distilled and of a dark sample which had been exposed to air for some time following distillation were dissolved in equal amounts of water and tasted. The solution of the old sample left an unpleasant bitter taste in the mouth. This was entirely absent in the case of solution of the freshly distilled sample. Another test was run on a mixture of furfuryl alcohol and its acetic and formic esters. These had been carefully redistilled and were fairly free from acid. After eight weeks the sample in an unsealed tube had become quite brown, that in a sealed tube under an air pressure of two millimeters was a very pale yellow, while that placed in an atmosphere having a 75 per cent humidity was a dark brown. Only the sample kept in a moist atmosphere had gone off appreciably in taste. It should be noticed that the relative stabilities of the three samples is the same as that of coffee kept under similar conditions. Coffee kept in a vacuum is the best preserved, that in air is not nearly so well preserved, and that in a moist atmosphere deteriorates very fast. If nearly an equal volume of acid had been added to these samples and they had been spread over a large surface, the differences and rate of deterioration would have been much greater. This is the condition in the coffee bean and especially in ground coffee after the carbon dioxide gas has had time to escape.

It is the similarity between the instability of impure furfuryl alcohol and of roasted coffee that makes us believe that these mixtures play an important part in the staleness of coffee. It is nearly impossible to single out one group of compounds in such a complex mixture as coffee and to say that they are responsible for its deterioration without first investigating other constituents. Repetition of this work on a somewhat larger scale will probably show up the presence of other easily changed materials. For instance the fraction suspected of being a phenol and reducing Tollen's reagent is very likely quite unstable in air and may be an important one of the group of compounds giving coffee its odor and taste. Some of the non-volatile constituents may also decompose in air to give products having un-

pleasant tastes and odors. At present we can say that we have isolated a mixture of compounds which are unstable in air and would seem to be in part responsible for the staleness of coffee, and we wish to point out the possibility of any group of compounds other than the fat being responsible for the staleness of coffee. This statement is based on experimental observations and not on pure speculation.

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DETECTION AND SIGNIFICANCE OF THE COLIFORM GROUP IN MILK[1] [2]

II. IDENTIFICATION OF SPECIES ISOLATED

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(Received for publication, January 15, 1937)

Comparatively few reports on the types of the coliform group present in milk have been published and in most instances the species have not been determined. It is difficult to compare earlier work with more recent investigations because of differences in classifications employed. Earlier work is summarized by Yale (1933). This investigator isolated 63 per cent *Escherichia*, 26 per cent *Aerobacter*, and 11 per cent intermediates from raw milk and 57, 10, and 33 per cent respectively from pasteurized milk. Ten species of *Escherichia* and three of *Aerobacter* were isolated by Yale, who found *Escherichia coli*, *Escherichia pseudocoloides*, and *Escherichia communior*, in this order, the most prevalent in the first genus, and *Aerobacter aerogenes* and *Aerobacter cloacae* most frequent in the latter. The results of more recent investigations will be included in the discussion.

EXPERIMENTAL WORK

The organisms identified in this report were isolated by inoculating 275 samples of raw milk in groups of 50 samples into a total of 12 media; 25 samples of raw milk into 10 media; and 31 samples of raw milk, 34 samples of pasteurized milk, and 25 samples of "certified" milk into five media as discussed in a previous paper, Bartram and Black (1936). The cultures were identified as *Escherichia*, *Aerobacter*, or intermediate according to their reaction on Methyl Red-Voges Proskauer medium, Simmon's citrate agar, and the indol reaction. The species in the *Escherichia-Aerobacter* genera were classified in accordance with the descriptions given in Bergey's Manual (1934). The species of the intermediate or *Citrobacter* genus were identified according to the classification of Werkman and Gillem (1932).

The technique and media employed in physiological tests were those recommended by the Committee on Bacteriological Technic of

[1] Approved for publication by the Director of the Maryland Agricultural Experiment Station.

[2] A portion of a thesis submitted to the Graduate School of the University of Maryland by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

the Society of American Bacteriologists in the Manual of Methods for Pure Culture Study of Bacteria (1934).

TABLE 1

Prevalence of Species of the Escherichia-Aerobacter Group Isolated from Milk

Genus	Species	Raw	Pasteurized	Certified
		310 cultures	15 cultures	8 cultures
		pct.	pct.	pct.
Escherichia	*communior*	20.0	.....	38.0
	coli	7.0	.....	.....
	paragruenthali	6.0	6.5	.....
	enterica	5.0	.....	.....
	formica	5.0	.....	.....
	gruenthali	4.5	.....	12.0
	communior (atypical)	2.0	.....	.....
	vesiculiformans	2.0	.....	.....
	pseudocoloides	1.6	.....	.....
	anindolica	1.6	.....	.....
	neapolitana	1.3	.....	.....
	anaerogenes	0.3	.....	.....
	leporis	0.9	.....	.....
Total		57.2	6.5	50.0
Aerobacter	*hibernicum*	12.0	.....	.....
	cloacae	5.0	6.5	.....
	aerogenes	3.0	.....	12.0
	oxytocum	1.6	.....	.....
	levans	0.6	.....	26.0
Total		22.2	6.5	38.0
Citrobacter	*freundii*	9.0	.....	12.0
	anindolicum	5.8	.....	.....
	decolorans	4.5	80.5	.....
	glycologenes	1.0	6.5	.....
	album	0.7	.....	.....
Total		21.0	87.0	12.0

RESULTS

Four hundred and eighty-four cultures were isolated from 331 samples of raw milk. Following identification of the species, duplicates from the same samples were eliminated and results reported were based on the remaining 310 cultures, as shown (Table 1). Of these, 176 or 57 per cent were *Escherichia;* 69 or 22 per cent, *Aerobacter;* and 65 or 21 per

cent, intermediates (methyl-red positive, Voges-Proskauer negative, and citrate positive). Cultures of *Escherichia* consisted of 12 species. The organisms in the *Aerobacter* genus comprised five species and the *Citrobacter* genus was made up of five species.

The pasteurized samples yielded 45 cultures of which 30 were duplicates. Of the 15 remaining, 1 (6.5 per cent) was *Escherichia*, 1 was *Aerobacter*, and intermediates comprised 13 (87 per cent). The *Escherichia* culture proved to be *Escherichia paragruenthali* and the *Aerobacter, Aerobacter cloacae*. The preponderance of one species is not surprising since the positive samples were all isolated from one pasteurizing plant where they probably resulted as contamination owing to improper cleaning or to faulty equipment.

Eight cultures were isolated from the "certified" milk. Four cultures were *Escherichia*, three *Aerobacter*, and one an intermediate type. Specifically these organisms were *Escherichia gruenthali* (1), *Escherichia communior* (3), *Aerobacter aerogenes* (1) and *Aerobacter levans* (2).

A portion of results showing the distribution of the species is shown (Table 2). There was apparently no influence by the different media on the species or genera obtained.

DISCUSSION

The preponderance of *Escherichia* over *Aerobacter* in raw milk (57:22) agrees in general with the results obtained by Yale (1933) and by Malcolm (1933), who found 71 per cent *Escherichia coli* in the winter and spring while in summer and autumn 40.4 per cent were *Escherichia coli*. Kon (1933) found *Escherichia coli* and *Aerobacter aerogenes* to occur equally in milk (58:42).

The frequent occurrence of *Escherichia communior* and *Escherichia coli* and *Aerobacter cloacae* and *Aerobacter aerogenes* agrees with the cited results of Yale. *Aerobacter hibernicum* was not identified by this investigator, since it is a new species, but he isolated several cultures similar to *Aerobacter cloacae* which failed to liquefy gelatin and possibly were *Aerobacter hibernicum*. Kline (1930) isolated only four species of *Escherichia* with *Escherichia neapolitana* the most common, and two species of *Aerobacter, Aerobacter cloacae* and *Aerobacter aerogenes*.

It would seem probable, from the frequent occurrence of two or more species in a given sample of raw milk, that the sources of contamination were varied and numerous. In pasteurized milk the regular appearance of one species would seem to indicate some one source of contamination and a possible harboring of the organisms at a point not reached by the cleaning process.

TABLE 2

Representative Portion of Results Showing Species Isolated from Individual Samples Raw Milk Inoculated into Four Media Simultaneously

Series											
I.				II.				III.			
Medium				Medium				Medium			
1	2	9	10	1	3	9	11	1	4	9	12
E_{14}	C_5	C_1	E_{14}	E_1	E_1	E_1	E_1	E_9	E_1	E_1	E_1
E_4	C_5	E_3	E_4	E_7	*E_{12}	*E_{12}	E_4	E_3	E_3	E_1	E_1
E	E	A_6	E_4	*E_{12}	C_2	E_{12}	C	E_4	E_2	E_1	*E_{12}
C_5	C_5	C_5	C_2	E_{16}	E	E_{12}	C_5	E_3	E_{12}	E_{12}	E_{12}
E_2	E_2	E_{12}	E_{12}	C_3	C_3	C_3	C_3	E_{12}	E_1	A_6	E_1
—	C_5	C_3	C_5	C_3	A_6	C_1	C_2	E_2	E_1	E_2	E_2
C_5	—	C_5	C_5	A_6	E_{12}	C_3	C	E_2	E_4	E_2	C_3
E_2	C_5	X	E_{17}	A_6	E_{12}	*E_{12}	A_6	E_1	E_1	E_1	E_{12}
C_7	A_1	—	—	A_4	A_4	A_4	C_3	E_3	E_3	E_3	C_1
*E_{12}	A_2	—	—	*E_{12}	*E_{12}	*E_{12}	A_6	—	E_{12}	E_{12}	E_{12}

Raw, Pasteurized and "Certified" Milk Inoculated into Five Media Simultaneously

Raw					Pasteurized					"Certified"				
Medium					Medium					Medium				
6	7	8	9	14	6	7	8	9	14	6	7	8	9	14
E_{12}	E_{12}	E_{12}	E_{12}	E_{12}	C_1	C_1	C_1	C_1	—	X	X	E_{12}	—	—
E_{12}	E_{12}	E_{12}	E_{12}	—	C_1	C_1	C_1	C_1	—	E_{12}	—	—	C_3	—
E_{10}	A_6	E_{10}	—	—	C_1	C_1	C_1	C_1	—	A_7	—	—	—	—
X	A_6	A_4	X	—	C_1	C_1	C_1	C_1	—	—	E_5	—	—	—
—	—	A_1	—	—	C_1	C_1	C_1	C_1	—	—	A_7	—	—	—

Raw Milk Inoculated into Ten Media Simultaneously

Medium									
1	3	4	5	6	7	9	10	11	13
A_1	A_1	A_1	A_1	A_1	A_1	A_1	A_1	A_1	A_1
E_{12}	E_{12}	E_{12}	E_{12}	E_{12}	—	E_{12}	E_{12}	—	E_{12}
—	A_6	A_6	A_6	E_{12}	E_{12}	A_6	A_6	X	E_{12}
A_6	A_{12}	X	A_6	E_{12}	E_{12}	C_1	A_6	X	X
E_{12}	—	E_{12}	E_5	E_{12}	X	E_5	E_{12}	X	E_{12}
X	—	E_3	E_3	E_3	E_3	E_3	—	X	X
E_{12}	—	X	E_1	—	—	E_{12}	—	—	—
E_1	X	E_1	—	A_6	—	—	—	—	—

MEDIA

1 Endo
2 Taurocholate
3 Ferrocyanide citrate
4 Brilliant green lactose bile
5 Neutral red bile
6 Violet red bile
7 Desoxycholate
8 Medium Four
9 Brilliant green bile
10 Methylene blue-brom cresol purple
11 Formate ricinoleate
12 Fuchsin lactose (old formulae)
13 Fuchsin lactose (modified)
14 Eijkman

E Escherichia
A Aerobacter
C Citrobacter
X False presumptive
— Negative presumptive
* Atypical (salicin negative)

Numbers refer to species according to Bergey (1934) or Werkman and Gillem (1932).

From the work of Kon, who found *Escherichia coli* to predominate 44:1 in bovine feces, it would appear that fecal contamination would be indicated by the presence of *Escherichia* only. However, the tendency to regard all members of the coli-aerogenes group as of possible fecal origin seems more in line with recent investigations. It will be noted from the small portion of individual samples included (Table 2) that there were some samples in which *Escherichia* were obtained with one or more media and not with other media which, however, often yielded other members of the *Escherichia-Aerobacter* group. This might in part explain the difference in the proportion of the various members of the group obtained by us and those obtained by other investigators. In addition, if *Escherichia* were to be used as the sole indicator of contamination, its presence might not be detected in all instances. This occurrence of two and occasionally all three members of the group simultaneously in a single sample of milk, indicates that the presence of any member of the group should be regarded as an indicator of contamination.

SUMMARY AND CONCLUSIONS

The examination of 331 samples of raw milk yielded 310 cultures of the *Escherichia-Aerobacter* group. These consisted of 57 per cent *Escherichia* composed of 12 species with *Escherichia communior* predominating; 22 per cent *Aerobacter*, five species, with *Aerobacter hibernicum* predominating; and 21 per cent intermediate, five species, *Citrobacter freundii* predominating.

With 34 samples of pasteurized milk from several plants cultures of *Escherichia-Aerobacter* were obtained from one. Of 15 cultures one was *Escherichia*, one *Aerobacter*, and 13 intermediates with *Citrobacter decolorans* predominating.

From 25 samples of "certified" milk eight cultures were obtained, consisting of four *Escherichia*, three *Aerobacter*, and one intermediate.

The *Escherichia-Aerobacter* group as a whole was more significant than any one genus or species in indicating contamination.

No correlation was observed between the different media used and the genus or species isolated.

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THERMOPHILIC CANNED-FOOD SPOILAGE ORGANISMS IN SUGAR AND STARCH

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(Received for publication, January 16, 1937)

Much work has been done on organisms present in sugar both from the standpoint of spoilage of sugar itself and its role in the spoilage of food products to which it is added. Canners have been interested in this particular problem and have insisted that refiners free their products of spoilage organisms. Many have done this, and sugar with few undesirable bacteria is now available to canners.

THERMOPHILIC BACTERIA IN SUGAR

Thermophilic bacteria causing trouble for the canner have been studied by many investigators, but papers by Cameron and Williams (1928, 1929), James (1927), Cameron (1930), Cameron and Bigelow (1931), Cameron and Yesair (1931), and Homans (1933) have discussed them from the standpoint of their origin in sugar. Owen and Mobley (1932) stated that more systematic improvement must be exerted by the refiner before these spoilage organisms will be eliminated from sugar.

Calton (1936) found that thermophilic anaerobes increased in number in the process of sugar production following filtration of standard syrup. Growth of flat-sour spoilage organisms was demonstrated in the pan storage tanks. Halverson (1936) examined 13 samples of sugar and found all of them to be within the limits allowed by the National Canners Association. Sulfide-spoilage organisms have been studied especially by Werkman and Weaver (1927) and later by Werkman (1929). Much sugar intended for use by canners of non-acid products is now examined for these organisms.

Thermophilic organisms have been divided into three groups according to the type of spoilage which they produce in canned foods. The groups are unlike in their physiological characteristics but have the property in common of growth at high temperatures and formation of resistant spores. Their presence in sugar was demonstrated about 1926, and the groups now recognized are flat-sour spoilage organisms, thermophilic anaerobes, and sulfide-spoilage organisms.

Standards have been proposed by the National Canners Association, given by Cameron (1930) and Bigelow (1931), for judging suit-

ability of sugar for canning. They have been valuable in helping to eliminate sugar which causes spoilage of the product to which it has been added. Since the method used for examining sugar for thermophilic spoilage organisms has been published by Cameron (1936), and by Tanner (1932), it will not be given here.

Ninety-one samples of beet and cane sugar were collected to determine the incidence of spoilage organisms at the present time.

TABLE 1

Thermophilic Spoilage Organisms in Commercial Samples of Sugar

Spores of bacteria causing flat-sour-spoilage limits of counts per 10 grams	Number of samples in class
0-75	72
75-150	13
Above 150	6
Tubes showing presence of thermophilic anaerobes	
0+ tubes 6—	77
1+ tubes 5—	8
2+ tubes 4—	5
6+ tubes 0—	1
Spores of bacteria causing sulfide-spoilage limits of counts per 10 grams	
None	69
0-5	16
5-10	3
10-15	1
Above 15	2

Twenty-five samples were secured during a canning season from a factory in which corn was being packed.

From the results given it appears that all three groups of spoilage organisms were present in the sugar samples (Table 1). Sulfide-spoilage organisms were demonstrated in 22 of 91 (24 per cent) samples. Applying standards proposed by Cameron (1930) of not more than five spores in 10 grams of sugar, seven samples (seven per cent) were not acceptable.

The samples appeared relatively less contaminated with spores of thermophilic anaerobes, for only 14 of 91 (15 per cent) samples contained these organisms. Of this group only one sample failed to meet the requirement of a satisfactory sugar. Great variation in counts of flat-sour spoilage organisms was shown by the various

samples. Cameron stated that the upper limit for flat-sour organisms is 75 spores per 10 grams of sugar. Using this standard 19 (20 per cent) samples failed to qualify as satisfactory sugar for canning.

THERMOPHILIC BACTERIA IN STARCH

While sugar has been quite carefully studied, starch has received less attention. It is occasionally added to foods, especially cream-style corn harvested in the earlier stages of maturity when there has not been sufficient starch formation to provide a smooth product. Shortly after demonstration of thermophilic bacteria in sugar, some speculation occurred as to their existence also in starch. Early methods for examination of starch were based upon Cameron's method for the examination of sugar and this, in general, is true of the methods which will be discussed here.

One of the first problems was the amount of starch to be examined. In the early work three per cent solutions in water, heated to 80°C. (176°F.) for five minutes with frequent stirring, were used. Several test media were then inoculated. Owing to the fact that a three-gram sample was so small tnat it might not be representative, the size of the sample was increased to 10 grams which were suspended in cold sterile water; desired amounts of this suspension were then added to the respective media as in the examination of sugar. These starch-media mixtures were then heated in the flowing steam sterilizer; thickening of the starch caused no trouble in later cultural work. In the early work many organisms which had no apparent relation to spoilage of canned foods appeared on the test media. Owing to unsatisfactory results with starch suspensions heated at 80°C.(176°F.) for five minutes, the heating period was increased to 15 minutes at 100°C.(212°F.).

Results indicate that starch may contain many thermophilic bacteria which spoil canned food (Table 2). Only two samples complied with the standards for sugar. These, however, were from lots which had been especially treated in the factory to rid them of spoilage bacteria. The remainder were secured on the open market or from canners and may have received no special attention. Of this group of samples only four had fewer flat-sour spoilage bacteria than 100 in 10 grams of starch. The highest number encountered in any one sample was 325 in 10 grams. Such data indicate that the number of spores of flat-sour-producing bacteria in most starch exceeds the limits; also, that it is possible to produce starch which will conform to the established standard. Thermophilic anaerobes also seem to be common in starch. Since only one-half as much starch is used as for examination of sugar, it appears that thermophilic anaerobes are fairly com-

mon contaminators. Sulfide-spoilage organisms were not as common as thermophilic anaerobes, since only about half of the samples contained them; many showed the presence of but one or two colonies in the entire series of six tubes. Organisms isolated from sulfite agar were studied at greater length. Not all of them were similar despite the fact that they were isolated from typical black colonies. They were placed in pea-decoction media in which 80 per cent of them gave typical characteristics of sulfide spoilage.

TABLE 2

Thermophilic Spoilage Organisms in Starch Samples Heated 15 Minutes at 100°C.(212°F.)

Sample No.	Flat-sour spoilage spores per 10 grams	Thermophilic anaerobes		Sulfide-spoilage spores per 10 grams
		Tubes +	*Tubes —*	
1	175	3	3	15
2	125	3	3	30
3	155	3	3	0
4	265	3	3	0
5	100	2	4	5
6	125	3	3	0
7	105	3	3	0
8	115	4	2	10
9	240	4	2	10
10	140	1	5	0
11	0	0	6	0
12	25	0	6	0
13	90	4	2	5
14	125	2	4	5
15	100	4	2	5
16	155	3	3	15
17	95	2	4	0
18	325	2	4	5
19	200	1	5	5
20	120	3	3	
Av. for 20 samples	139	....	....	5.5

Results obtained on this limited number of samples, in general, confirm those reported by Owen and Mobley (1932), who found the average number of flat-sour producing bacteria in samples of starch they examined to be 131.9 per 10 grams of starch, 50 per cent of the samples showing thermophilic anaerobes and 33 per cent, hydrogen-sulfide producers. These results indicate that starch may contain relatively large numbers of spoilage organisms.

To study effect of starch with varying bacterial content on food products, cans of whole-grain corn were first incubated to prove absence of spoilage organisms of the types in starch. The brine was

removed and the corn placed in sterile cans; these were heated to 100°C.(212°F.) in the Arnold sterilizer, and sterile brine containing five per cent of the raw starch heated to 80°C.(176°F.) was added. The cans were then reheated, closed at a temperature of 90°C. (194°F.), and processed at 119°C.(246°F.) for 70 minutes. Care was taken to see that everything was sterile except the test starch solution. Eight different samples of starch were used and the cans were packed in duplicate. After 10 days' incubation at 55°C.(131°F.) none of the cans had swelled. The vacuum of the cans ranged from two to six inches and the pH was 6.4 when the cans were opened. There was no indication of spoilage by flat-sour producing or sulfide-spoilage organisms in any of the cans. In but one case did the starch used meet the standard for flat-sour producing organisms as established by Cameron for sugar. Even in the cans to which starch with a flat-sour

TABLE 3

Spoilage Organisms in Starch Samples Heated at 100°C. for Varying Lengths of Time

Time heated	Number of samples	Average count of flat-sour organisms per 10 grams	Average thermophilic anaerobes per 10 grams		Average sulfide spoilage organisms per 10 grams
min.					
15	10	107	7+	5—	6
30	10	20	6+	6—	2
45	10	16	4+	8—	2
60	10	21	6+	6—	2

count of 325 bacteria per 10 grams had been added, no spoilage occurred. Though only a limited number of samples of starch were tested in this work, lack of spoilage even from what appeared to be badly contaminated starch led to further work.

Study was then given the method for examining starch. Experience with sugar had indicated that practically no change in count of spoilage organisms resulted when the solution was heated longer than five minutes. Since no spoilage resulted in the canned corn, it was deemed advisable to test the heat resistance of organisms in starch. Consequently samples were rechecked and 15 minutes of heating at 100°C. was compared with 30, 45, and 60 minutes at that temperature (Table 3).

The results for thermophilic anaerobes and sulfide-spoilage organisms represent the counts obtained from a series of 12 tubes rather than the series of six usually employed.

It appears that the longer period of heating at 100°C. very materially decreased the number of organisms appearing in the culture

media as spoilage organisms (Table 3). This is most apparent with flat-sour organisms and those classified as sulfide-spoilage organisms. All of the samples tested meet the standard established by Cameron for sugar samples when the longer heating period was used. Some of the organisms producing black colonies in sulfite agar heated for 15 minutes must not have been *Clostridium nigrificans.* According to results reported by Werkman (1929) spores of this organism will resist boiling temperature for periods of 450 to 480 minutes in a neutral solution. Spores of this organism would not be destroyed by 30 minutes' heating at 100°C. Thermophilic anaerobes seem to be least affected by increased heating periods. Results reported in the above table seem to justify a period of heating of at least 30 minutes at 100°C. The method finally adopted and the one which is now proposed is as follows:

Preparation of Samples: Ten grams of carefully mixed starch are weighed into a sterile flask. To this is added, with stirring, enough sterile water to make the weight up to 100 grams. This mixture is then thoroughly stirred and pipetted into the media used for determining the various groups of spoilage organisms.

Flat-Sour Organisms: Twenty c.c. of the previously prepared starch suspension are pipetted into 100 c.c. of melted dextrose brom-cresol-purple agar or tryptone brom-cresol-purple agar, the mixture being stirred as the starch suspension is added. This agar and starch mixture is then heated with shaking until the starch flows and is then placed in an Arnold sterilizer for 30 minutes with occasional stirring: after this it is distributed equally among six Petri dishes. Incubation was at 55°C.(131°F.) for 36 to 48 hours.

Thermophilic Anaerobes: Twenty c.c. of the above starch-water mixture are equally distributed among six tubes of liver-infusion broth which have been previously heated to 100°C.(212°F.). After reheating and agitation to distribute the starch thoroughly and prevent excess foaming, they are placed in an Arnold sterilizer at 100°C. for 30 minutes, cooled, and stratified with plain agar. They are then incubated at 55°C. for five days.

Sulfide-Spoilage Organisms: Twenty c.c. of the starch suspension are distributed equally between six tubes of melted "sulfite agar"; the medium and starch are shaken thoroughly to distribute starch and then heated in the Arnold sterilizer for 30 minutes at 100°C. After heating, the medium is again thoroughly stirred, allowed to harden, and incubated at 55°C. for five days.

Yesair[1] of the National Canners Association has adapted the procedure for determining canned-food spoilage bacteria in sugar to

[1] Private communication.

starch. Since it has not been published, it will be presented as briefly as possible here. Also four different modifications of procedure for determining the presence of canned-food spoilage bacteria in starch have been privately reported by Yesair; they were compared in this investigation. It is unnecessary to review them in detail. In general various procedures have some basis in the method for sugar. The thickening of starch when heated makes the unmodified use of the sugar method impossible. The main differences in the proposed methods for determining the numbers of flat-sour spoilage organisms are in the amount of starch used and the manner and length of time

TABLE 4

Brief Description of Methods for Preparation of Starch Samples Proposed by Yesair

Method	Amount of starch in test	Treatment of sample	Method of heating	Heating temp.	Time
	gm.			*°F.*	*min.*
2	.5	Starch added to water, heated, mixture added to media.	Water bath	212	6
3	2.0	Starch and water mixture boiled in beaker, then double strength agar added, and plates poured.	Boiling	212	5
4	2.0	Starch and water mixed, added to agar, and heated.	Water bath	212	20

of heating before culturing. Brief description of methods collected by Yesair is shown (Table 4).

The following method designated as Method 6, reported to us as now being used by the National Canners Association, is a modification based upon experience with various methods described above. In the main it is based upon Method 4 and the proposed method. It differs from the one proposed in this paper in the testing of a greater amount of starch for thermophilic anaerobes and sulfide-spoilage spores and in the manner of heating the sample.

Preparation of Sample: Place 20 grams of starch in a dry, sterile, 150-c.c. Erlenmeyer flask and add sterile cold water to the 100 c.c. mark, with intermittent shaking. Shake well to obtain a uniform suspension of the starch in water.

Detection of Flat-Sour Spores: Pipette 10 c.c. of the starch suspension into 100 c.c. tryptone agar at a temperature of 54.4 to 60°C.(130 to 140° F.). Use

large bore pipettes and keep the starch suspension under constant agitation during the pipetting operation. After the starch has been added to the tryptone agar, shake the flask in boiling water for a period of three minutes to thicken the starch. Then place the flask in the autoclave and heat at five pounds' pressure for 10 minutes. This is suggested as a means of cutting down the time of heating. After autoclaving, the flask should be gently agitated while cooling. Violent agitation will incorporate air bubbles in the medium which may subsequently interfere with the reading of the plates. When the agar-starch mixture is cooled to the proper point, pour five plates and allow them to harden to a maximum degree. When the plates have hardened, stratify with a thin layer of plain two-per cent agar. The agar should contain no nutrient; it is added for the purpose of combating the "spreader" difficulty. Comparative tests that were made in this laboratory indicate that this procedure is effective in cutting down the number of spreaders. The effect of stratification on flat-sour counts, however, has not as yet been tested. It is expected, however, that stratification will have nothing more than a minor effect on the flat-sour count. Incubate the plates at 55°C. (131°F.) and make readings in 24 and 48 hours.

Detection of Thermophilic Anaerobes not Producing H_2S: Divide 20 c.c. of the cold-starch suspension approximately equally among six liver-broth tubes. These tubes should be spun before heating and during the heating period. The method of spinning the tubes and the time of heating will be discussed later. In the liver-broth tubes, it has been found desirable to use finely ground liver in very small amounts. The fine liver will, when the starch thickens, remain in permanent suspension throughout the medium and this is considered an advantage. It appears to be unnecessary to stratify the liver-broth tubes.

Detection of Thermophilic Anaerobes Producing H_2S: Divide 20 c.c. of the cold-starch suspension approximately equally among six sulfite-agar tubes. These tubes also should be spun before heating and during the heating period.

The heating and spinning of the tubes in this laboratory is done in a specially built water bath that may be described as follows:

The bath is constructed of heavy galvanized iron and has a length of 13½ inches, width 6½ inches, depth 4 inches. The top of the bath has two rows of 12 holes each. These holes are ¾ inch in diameter and approximately one inch apart from center to center. The centers of the holes are approximately 1¼ inches from the edge of the bath. At one end of the top there is a large hole 1¼ inches in diameter for the purpose of replenishing the water. The top is removable with ½-inch side flanges fitting over the outside of the body.

The bath also contains a false bottom supported one inch from the bottom of the bath with one-inch side flanges. The bottom contains the same number of holes and is the same size as the top. These holes are made to coincide vertically with the holes in the top. In addition, 14 small holes ¼ inch in diameter are placed between the rows of large holes for the purpose of permitting proper circulation.

Rotation or spinning is performed by means of two rubber covered wooden blocks. These blocks are 13½ inches by 1½ inches by ½ inch. They are equipped with center uprights which serve as handles. The tubes are spun between the rubbered surfaces. Twelve tubes may be spun at one time.

In inoculation and heating the liver-broth and sulfite-agar tubes it is quite desirable to maintain an even suspension of the starch, and for this purpose it is necessary to agitate them at frequent intervals. In this laboratory the tubes are spun or agitated as follows:

(1) The tubes are spun in the hands, three at a time, just after the addition of the unheated starch.

(2) After the tubes are placed in the water bath the individual tubes are spun with the fingers.

(3) After all tubes are in the bath they are spun mechanically with the previously described blocks three or four times during the first five minutes of heating. After this, the heating is continued for 10 minutes.

(4) After the liver and sulfite tubes have been heated they are placed in cold water to solidify. The tubes are preheated to 55°C. and incubated at that temperature for 48 hours.

Reporting Results: Report total counts and flat-sour and sulfide-spoilage results as number of spores per 10 grams of starch. Report thermophilic anaerobes (not producing H_2S) as number of tubes positive and number negative in the following manner: + + +, — — —.

Standards

The same standards for the thermophilic-spore counts as used for sugar are applied to starch. They are as follows:

Total Thermophilic-Spore Count: For the five samples examined there shall be a maximum of not more than 150 spores and an average of not more than 125 spores per 10 grams of starch.

Flat-Sour Spores: For the five samples examined there shall be a maximum of not more than 75 spores and an average of not more than 50 spores per 10 grams of starch.

Thermophilic Anaerobic Spores: These shall be present in not more than three (60 per cent) of the five samples and in any one sample to the extent of not more than four (65+ per cent) tubes.

Sulfide-Spoilage Spores: These shall be present in not more than two (40 per cent) of the the five samples and in any one sample to the extent of not more than five spores per 10 grams. This would be equivalent to two colonies in the six inoculated tubes.

Results secured by the proposed method were compared with those secured with other methods (Table 5).

The counts reported here are only typical colonies of flat-sour spoilage organisms. Where no count is recorded, as in Methods 2, 3, and 4, typical colonies were not seen on the plates. These plates showed larger numbers of colonies but none produced acidity typical of flat-sour spoilage organisms. Difficulty in counting was often due to overgrowth of spreading colonies. The proposed method and Method 6, while showing the presence of spreaders, were not so prone to be overgrown by them. Yesair has suggested stratified plates as a means of inhibiting spreaders.

It will also be observed that in many cases Methods 2, 3, and 4 gave much higher counts than the other two. It is undoubtedly true that larger samples are more representative and probably give more accurate results. Too-short heating periods do not destroy spores of low resistance which are of little significance in food spoilage but which do contribute to high counts.

Samples 8 and 9 were made as an experimental run. The appropriate amount of sterile starch was added to the media and then a spore suspension of a typical flat-sour spoilage organism (No. 1518) was added. Sample 8 represented the original spore suspension; Sample 9, a one-to-ten dilution of this same suspension. The respective methods were then used and results tabulated. All organisms developed on the plates were counted, since care was taken to see that only the test organism was added. Count of the suspension indicated that approximately 1,450 flat-sour organisms had been added to Sample 8, and 140 to Sample 9. In this test Method 2 and the proposed method checked closely with the numbers of organisms added. Method 6 gave slightly lower counts than expected and Method

TABLE 5

Comparison of Numbers of Flat-Sour Spoilage Organisms in Starch by Different Methods

Sample No.	Method 2	Method 3	Method 4	Proposed Method	Method 6[1]
1	0	27	0	35	10
2	1230	88	0	20	20
3	550	....	260	25	20
4	290	90	420	27	20
5	10	0	55	7	2
6	0	0	177	2	0
7	50	30	152	52	17
8	1460	360	1960	1510	1125
9	132	97	144	144	129

[1] Proposed by Yesair.

4, higher counts. Method 3 proved to be the poorest of the five methods tried.

The proposed method and Method 6 gave consistently better results than any of the others suggested (Table 5). They gave clearer cut results and were less erratic than the others. Method 4 gave consistently higher results. In many cases using Methods 2, 3, and 4 it was evident that the organisms developing were not those of the flat-sour type.

There is little difference in the method proposed for the determination of thermophilic anaerobes and sulfide-spoilage organisms in starch and sugar. It is essential that the starch be heated carefully since it tends to foam and moisten the cotton stoppers.

Results secured indicate that all the methods gave approximately equivalent results (Table 6). When 20 grams of starch were added to 100 c.c. of water and then 20 c.c. of this suspension added to tubes of liver infusion, difficulty was encountered in heating at 100°C. (212°F.) for any period of time because the bubbles which were

formed caused the entire mixture to rise in the tube and foul the plugs. Almost continual agitation was necessary to prevent this. When 20 c.c. of a water suspension of 10 grams per 100 c.c. were used, the mixture in the liver broth was not nearly so prone to do this. The objection to this latter proposal is the small amount of starch used. However, when relatively large numbers of thermophilic anaerobes or

TABLE 6

Incidence of Thermophilic Anaerobic Bacteria in Starch Determined by Different Methods

Method	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
4	2+ 4—	....	6+	5+ 1—	5+ 1—	....	0 6—	....
	2+ 4—	....	5+ 1—	4+ 2—	6+	....	0 6—	....
Proposed method	2+ 4—	4+ 2—	5+ 1—	5+ 1—	6+ 0	6+	0 6—	5+ 1—
	2+ 4—	4+ 2—	5+ 1—	5+ 1—	6+ 0	6+	0 6—	5+ 1—
6	3+ 3—	5+ 1—	6+	4+ 2—	6+ 0	6+	0 6—	5+ 1—
	3+ 3—	4+ 2—	4+ 2—	5+ 1—	6+ 0	6+	0 6—	4+ 1—

sulfide-spoilage organisms are present, this objection is not so valid. In the few tests run so far this latter method has proved as efficient as when larger amounts are used.

One of the difficulties in the test for sulfide-spoilage organisms is the ability of many anaerobes to produce hydrogen sulfide. They later produce gas sufficient to break up the medium, indicating that

TABLE 7

Incidence of Sulfide-Spoilage Bacteria in Starch Determined by Different Methods

Method	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
4	....[1]	....	....	14	....	....	....	....
				24		....		
Proposed method	10	15	10	13	15	5	0	0
				11		6		
6	5	10	10	8	10	5	0	0
				15		5		

[1] Count was questionable because of breaking up of agar after first production of black colony.

they are not typical organisms of this group. Observation on marked colonies in this test proved this. This precaution is especially applicable to samples containing thermophilic anaerobes in abundance. A slightly longer incubation period than 72 hours seems advisable for their detection. Results on the samples of starch tested for sulfide-spoilage organisms are shown (Table 7).

Method 4 especially developed atypical sulfide-spoilage organisms. In one sample typical black colonies appeared in 72 hours, but 36 hours later more than half of them showed breaking of media owing to gas production where originally only the black colony appeared. This is overcome in part by the longer heating period proposed in Method 6 and the proposed method. Here there is less tendency for this to occur and the results give nearer to a true count of sulfide-spoilage organisms.

SUMMARY

All three groups of thermophilic spoilage organisms were found in samples of sugar, though in the majority of cases they were not present in excessive numbers. Few samples were free from members of all three groups. The flat-sour spoilage organisms were the most prevalent type found. These results indicate that considerable improvement has beeen made in production of sugar of good bacteriological quality. A method for examining starch for thermophilic spoilage organisms is proposed and was compared with other methods. All of these methods were modifications of Cameron's method for analysis of sugar. Because of the thickening property of starch, modifications have been necessary. It has been observed that a longer heating period is necessary for destruction of spores of non-spoilage organisms in starch than in sugar. This is best accomplished by preparing a cold-water suspension of the starch, adding it to the hot media, and then carefully heating this mixture. The method proposed in this paper and that by Yesair gave the most consistent results. Although members of all three groups of thermophilic spoilage organisms were present, thermophilic anaerobes were most abundant in the starch samples tested.

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STABILITY OF VITAMIN C IN SWEET CORN TO SHIPPING, FREEZING, AND CANNING

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(Received for publication, January 17, 1937)

The only reference found in the literature relative to vitamin-C content of sweet corn is by Rose (1931), who stated that fresh sweet corn contained an amount of vitamin C that is of significance in nutrition. Sweet corn is used as a food the year round either fresh, frozen, canned, or even dried. Because of this abundant use of sweet corn for human consumption, it seemed desirable to determine its content of vitamin C.

EXPERIMENTAL PROCEDURE

Fresh Golden Bantam sweet corn from the Massachusetts State College experimental plots was examined (raw and after being cooked) for ascorbic-acid content by the Tillmans, Hirsch, and Hirsch titration method (1932), as modified by Bessey and King (1933). Golden Bantam sweet corn was quick-frozen at the New York and Maine factories of the Birds Eye Frosted Foods Sales Corporation and shipped to Amherst. Simultaneously, iced shipments from the same lots of freshly picked sweet corn were made semiweekly to Amherst while the corn season lasted. At the same time a third portion of the same sweet corn was canned and later shipped to Amherst. In this manner information was obtained concerning the ascorbic-acid (vitamin-C) content of fresh, frozen, and canned sweet corn. Samples were titrated for ascorbic-acid content by the 2, 6-dichlorophenolindophenol dye, and animal assays on guinea pigs by the Sherman method (1922) were also carried out.

In addition to the canned sweet corn from the Frosted Foods Sales Corporation, 11 different brands of canned sweet corn were examined for ascorbic-acid content. Animal assays were carried out on one of these brands, a white, cream-style sweet corn.

Since during the corn season much fresh sweet corn is bought from the market rather than fresh from the field, 12 samples of market sweet corn in the husk were examined for ascorbic-acid content and compared with fresh sweet corn.

The effect of heat on the stability of vitamin C in sweet corn was determined by (a) cooking fresh, raw sweet corn in water for 12 minutes, (b) cooking frozen sweet corn in water for 4 minutes, and (c) reheating canned sweet corn for 15 minutes.

Storage tests on raw, fresh sweet corn in the husks were carried out at room temperature; at refrigerator temperature 4.4°C.(40°F.); and with blanched, raw, fresh, whole-kernel sweet corn at —23.3°C. (—10°F.). Further storage tests were carried out with canned sweet corn removed from the can and exposed to the air at 4.4°C. Frozen, whole-kernel sweet corn and frozen sweet corn-on-cob were titrated for ascorbic-acid content upon arrival in Amherst; then they were

TABLE 1

Comparison of Dye-Titration and Animal-Bioassay Methods for Determination of Ascorbic Acid in Sweet Corn

Golden Bantam variety	Number of guinea pigs	Protective level for guinea pigs	Titration value for ascorbic acid	Number of samples	Calculated protective level [1]	Estimated vitamin-C content
		gm.	*mg. per gm.*		*gm.*	*Internat. units per oz.*
Fresh, raw	3	6.5	.09	10	5.6	51
Fresh, cooked	3	8.5	.08	10	6.2	45
Frozen	3	9.0	.08	10	6.2	45
Cream-style, canned	3	14	.07	6	7.1	40
Whole-kernel, canned	....	.....	.08	5	6.2	45
Iona brand, white cream-style, canned [2]	3	12.5	.08	15	6.2	45

[1] Based on daily requirement of .5 mg. ascorbic acid per guinea pig. [2] A commercial canned product, not comparable to the others listed in Table 1.

stored at —23.3°C.(—10°F.) for 35 days and again titrated for ascorbic-acid content. Storage data are presented (Table 2.)

Determinations were also made of the loss of ascorbic acid during defrosting of frozen sweet corn.

The variation of ascorbic-acid content during the harvesting season was studied with Amherst-grown, fresh sweet corn over a period of 50 days.

COMPARISON OF DYE-TITRATION AND ANIMAL-BIOASSAY METHODS

Satisfactory checks were obtained with fresh-raw and fresh-cooked sweet corn, but considerable deviation between dye titration and animal assay resulted in the case of frozen and canned sweet corn (Table 1). The only explanation which seems tenable is that losses

in ascorbic acid occurred before the guinea pigs ate the relatively large quantities fed them. It hardly seems probable that freezing or heat could liberate other dye-reducing substances in addition to ascorbic acid. At any rate definitely higher results were obtained by the dye method.

The data show that sweet corn, either fresh-cooked or frozen-cooked, is a fair source of vitamin C and that while canned corn is more variable, many brands must also be considered fair sources of this vitamin. The samples (Table 1) are comparable in that they were all from the same lot of sweet corn. Cream-style canned corn contained somewhat less vitamin C than whole-grain corn. This seems

TABLE 2

Loss of Ascorbic Acid in Sweet Corn During Storage
(Milligrams per gram)

Treatment	Number of days' storage					
	0	1	2	3	4	34
Fresh corn in the husks stored in refrigerator at 4.4°C.(40°F.)	.14	.12	.10	.09	.....	.....
Fresh corn in the husks stored at room temp., 18 to 27°C.(65 to 80°F.)	.14	.13	.12	.08	.07	.....
Fresh corn, blanched, stored at —23.3°C.(—10°F.)	.05	.....	.....	.....	.....	.06
Frozen, whole-kernel corn stored at —23.3°C	.10	.....	.....	.....	.....	.10
Frozen corn-on-cob stored at —23.3°C	.10	.....	.....	.....	.....	.10
Iona, white, canned corn, stored in refrigerator after opening, 4.4°C	.08	.08	.07	.06	.04	.....
A & P, yellow, canned corn, stored in refrigerator after opening, 4.4°C	.07	.07	.07	.06	.05	.....

reasonable in view of the greater opportunity for oxidation in the former.

EFFECT OF STORAGE OF SWEET CORN ON ITS VITAMIN-C CONTENT

Since sweet corn is often held for several days in the husk after picking, the effect of storage was determined. Freshly picked samples of Golden Bantam variety were stored at both room and cold-storage temperatures and titrated for ascorbic-acid content from time to time. The data given show that fresh sweet corn may be stored even at room temperature for 24 hours with less than 10 per cent loss in vitamin-C content, and that in four days' storage the loss is less than 50 per cent (Table 2). The iced shipments of corn-in-husk from Albion, New

York, to A erst, Massachusetts, requiring about one to two days in transit, snowed average values of .10 mg. of ascorbic acid per gram, indicating small shipping loss.

Exposure of canned sweet corn, after opening the can, had little effect on vitamin C even after three days' storage in the refrigerator at 4.4°C.(40°F.). When either fresh, whole-kernel corn or corn-on-cob was blanched to set the milk and stored at —23.3°C.(—10°F.) for 34 days, no loss occurred. It is apparent from this work that the vitamin C in sweet corn is relatively stable in so far as storage is concerned.

EFFECT OF FREEZING

When freezing sweet corn it is necessary to blanch it in steam or hot water for a few minutes to set the milk and inactivate enzymes. That freezing had no effect on vitamin C in sweet corn was demonstrated many times. Ten samples showed no essential change in freezing (Table 1). However, in blanching and other operations incidental to commercial freezing some slight loss may occur. During the blanching process a small amount of vitamin C passes into the water and is lost. In steam blanching very little loss occurs.

Ten samples of Birds Eye, frozen, whole-kernel, Golden Bantam sweet corn showed a maximum value of .10 mg. ascorbic acid per gram, a minimum value of .05, and a mean value of .08 or 45 international units per ounce. The average of three samples of Birds Eye, frozen, Golden Bantam corn-on-cob was .10 mg. per gram or 56 units of vitamin C per ounce. The average value for frozen, whole-grain, Country Gentleman corn was .09 mg. ascorbic acid per gram. As stated previously, no loss occurred in the vitamin-C content of either frozen corn-on-cob or whole-grain corn while stored at —23.3°C. (—10°F.).

EFFECT OF DEFROSTING FROZEN SWEET CORN

Samples of whole-kernel, Golden Bantam sweet corn were solidly frozen at —23.3°C. and gradually defrosted at refrigerator temperature, 4.4°C.(40°F.). Complete defrosting required 24 hours. The corn lost 1.5 per cent of its vitamin C in six hours and only three per cent after 24 hours. Complete defrosting at room temperature showed a loss of only six per cent. Apparently, frozen sweet corn need not be reheated while still frozen in order to conserve its vitamin-C content. Peas and lima beans lose much of their vitamin C on defrosting, according to Fellers and Stepat (1936).

EFFECT OF COOKING AND CANNING

Normally, sweet corn-on-the-cob is boiled before it is eaten; frozen and canned corn are likewise heated before serving. Some experiments

on the effect of heat on vitamin C in sweet corn were conducted and the results summarized (Table 4). Fresh-raw and frozen sweet corn were heated in an open kettle until ready to eat. The loss of ascorbic acid was respectively 6.5 and 6.8 per cent. Canned yellow and canned white sweet corn were also examined for ascorbic-acid content before

TABLE 3

Vitamin-C Content of Commercially Canned Sweet Corn

Brand	Titration value for ascorbic acid	Vitamin C per ounce
	mg. per gm.	*Internat. units*
Iona, white, cream-style	.08	45
Del Monte, cream-style	.10	57
Premier Fancy, cream-style	.07	40
Del Maiz, cream style	.09	51
A. & P., yellow, cream-style	.07	40
Premier Fancy, whole-kernel	.09	51
B. & M., whole-kernel	.07	40
Sweet Life, whole-kernel, vacuum-packed	.10	57
Niblets, whole-kernel, vacuum-packed	.10	57
Del Monte, whole-kernel, vacuum-packed	.10	57
Collins' Hulled, whole-kernel	.03	17

and after reheating. Losses in ascorbic acid of 8.7 and 4.6 per cent respectively were recorded as due to the heating. The canned corns undoubtedly would lose even less than this if they were heated in

TABLE 4

Effect of Heating on Ascorbic-Acid Content of Sweet Corn

Treatment	Unheated	Heated [1]	Loss due to heating
	mg. per gm.	*mg. per gm.*	*pct.*
Fresh corn-on-cob, raw, cooked 12 min. in boiling water	.089	.083	6.7
Frozen whole-grain corn, cooked 4 min. in boiling water	.082	.076	7.3
Canned white corn, reheated to boiling point for 1 min.[2]	.080	.076	5.0
Canned yellow corn, reheated to boiling point for 1 min.[2]	.070	.064	8.6

[1] All corn heated until ready to serve. [2] Contents removed from the can for reheating.

the unopened cans. The loss of ascorbic acid in heating sweet corn until ready for the table is slight, amounting to less than nine per cent.

There are so many brands of canned sweet corn on the market that it seemed expedient to determine the ascorbic-acid content of several of them. It is evident that cream-style, canned sweet corn

contains an average of .08 mg. per gram of ascorbic acid, and whole-kernel, canned sweet corn has an average of .09 mg. ascorbic acid per gram (Table 3). Vacuum-packed, canned sweet corn averages over .10 mg. ascorbic acid per gram of corn. Apparently the process of "creaming" the canned corn exposes a large area of it to oxidation and results in a loss of ascorbic acid; also the process of obtaining a vacuum by heat exhaust destroys more ascorbic acid than the mechanical exhaust. In the case of Collins' hulled corn, the lye treatment which the corn undergoes seems to destroy a considerable percentage of its ascorbic acid. In general whole-kernel, canned sweet corn has a higher ascorbic-acid content than has the cream-style. It is possible

TABLE 5

Variation of Ascorbic-Acid Content of Fresh Sweet Corn During Corn Season [1]

Date harvested and examined	Titration value for ascorbic acid
	mg. per gm.
8/15/36	.09
8/17/36	.12
8/27/36	.10
9/ 4/36	.07
9/10/36	.08
9/18/36	.09
9/26/36	.08
10/ 1/36	.06
10/ 6/36	.07

[1] All samples were taken at approximately the same stage of maturity and from the same field. Each value represents three determinations.

that vacuum-packing also aids in preserving the ascorbic-acid content, though the data are not sufficient to make deductions.

In general canned sweet corn sold on the market is a fairly good source of vitamin C, and some brands are equal to fresh sweet corn.

ASCORBIC-ACID CONTENT OF SWEET CORN DURING THE HARVESTING SEASON

Fresh sweet corn from the Massachusetts State College field plots was examined from time to time throughout the harvesting season, August 15 to October 6, for ascorbic-acid content. There proved to be considerable variation (Table 5). The ascorbic-acid content of the sweet corn harvested the first month averaged .09 mg. per gram, while during the second month the average ascorbic-acid content was but .07 mg. per gram. From these limited data it may be assumed that sweet corn picked early in the season has a somewhat higher ascorbic-acid content than that picked later in the season. The decline of

ascorbic acid in the sweet corn during the two months' harvesting season was about 15 per cent.

ASCORBIC-ACID CONTENTS OF FRESH FIELD CORN AND MARKET CORN

Raw, fresh sweet corn varied in ascorbic-acid content from a minimum of .06 to .14 mg. ascorbic acid per gram with a mean value of .09 mg. per gram for 10 samples analyzed.

Market corn as purchased on the Amherst markets had a mean value of .09 mg. ascorbic acid per gram of corn for 10 different samples taken on different days, with a minimum of .012 and a maximum of .13 mg. per gram.

From these data it may be assumed that, on the whole, market corn compares favorably in vitamin-C content with freshly picked sweet corn.

VITAMIN-C CONTENT OF RAW, COOKED, FROZEN, AND CANNED SWEET CORN DETERMINED BY GUINEA-PIG BIOASSAY

The samples described (Fig. 1 and Table 6), with the exception of Iona Brand canned sweet corn, are strictly comparable in that parts of the same lot of sweet corn were cooked, frozen, and canned for use in this investigation. It may be seen that 7.5 grams of raw, fresh sweet corn, 10 grams of cooked sweet corn, and 10 grams of frozen sweet corn each contained sufficient vitamin C to protect a guinea pig from scurvy (Fig. 1). Twelve and a half grams of canned sweet corn used in this experiment, although showing almost as high a content of ascorbic acid by the titration method (Table 1) as frozen corn, did not give full antiscorbutic protection to the guinea pigs. This discrepancy may be explained as being due to the oxidation of the ascorbic acid of the corn in the feed dishes before the guinea pigs consumed all of it. A delay of one to six hours occurred in some cases, though the usual exposure time was 20 to 40 minutes. However, the above statement holds only when large amounts of the corn were fed to the guinea pigs. An actual determination showed that canned, cream-style sweet corn exposed in feed dishes for six hours lost 20 per cent of its ascorbic-acid content. Under the same conditions frozen, whole-kernel sweet corn showed no loss at all.

The data obtained in all the sweet corn bioassays are largely self-explanatory (Table 6). It will be seen that all the levels of sweet corn fed as fresh-raw, fresh-cooked, and frozen sweet corn contained sufficient vitamin C to prevent fatal scurvy over the period of time that the experiment lasted. The fresh sweet corn was available for feeding for only about two months. Although with the yellow canned sweet

TABLE 6

Summary of Vitamin-C Bioassays on Golden Bantam Sweet Corn

Description	Months fed	Quantity fed daily	Period of bioassay	Average survival period	Average total weight gains	Sherman scurvy score	Probable protective level
		gm.	*days*	*days*	*gm.*		*gm.*
Fresh, raw	Aug.-Oct.	5	65	65	11	6, 5, 4	
Fresh, raw	Aug.-Oct.	7½	52	52	196	0, 0, 0	6 - 7
Fresh, raw	Aug.-Sept.	10	54	54	207	0, 0, 0	
Fresh, cooked	Aug.-Oct.	5	60	59	—1	0, 2, 3	
Fresh, cooked	Aug.-Oct.	7½	52	52	30	½, ½, 5½	8 - 9
Fresh, cooked	Aug.-Sept.	10	45	45	78	0, 0	
Frozen	Sept.-Dec.	5	90	90	114	7, 2, 2	
Frozen	Sept.-Dec.	7½	90	90	63	6½, 6, 0	8.5 - 9.5
Frozen	Sept.-Dec.	10	90	90	215	0, 0, 0	
Yellow, canned	Aug.-Nov.	7½	90	50	—70	15, 17½, 19	
Yellow, canned	Sept.-Dec.	10	90	90	39	5, 7, 7	14 - 15
Yellow, canned	Aug.-Nov.	12½	90	90	142	2, 3, 7	
White, canned[1]	Aug.-Nov.	7½	90	50	—55	7, 9, 16	
White, canned[1]	Aug.-Nov.	10	90	75	63	3½, 6, trace	11 - 12
White, canned[1]	Aug.-Nov.	12½	90	90	131	0, 0, 0	

[1] Not Golden Bantam variety.

corn the guinea pigs showed a substantial weight gain, this amount of sweet corn was insufficient to prevent scurvy for 90 days. It is certain that the protective levels determined by the Sherman assay method (Table 6) represent maximum rather than minimum values. This is particularly true where large quantities of corn were fed.

Apparently sweet corn does not contain reversibly oxidized (dehydro-) ascorbic acid because several tests made after reduction with hydrogen sulfide showed no increases in titratable ascorbic acid. The authors feel that the dye-titration tests reported in this paper more nearly represent the amount of ascorbic acid present than the animal-bioassay test.

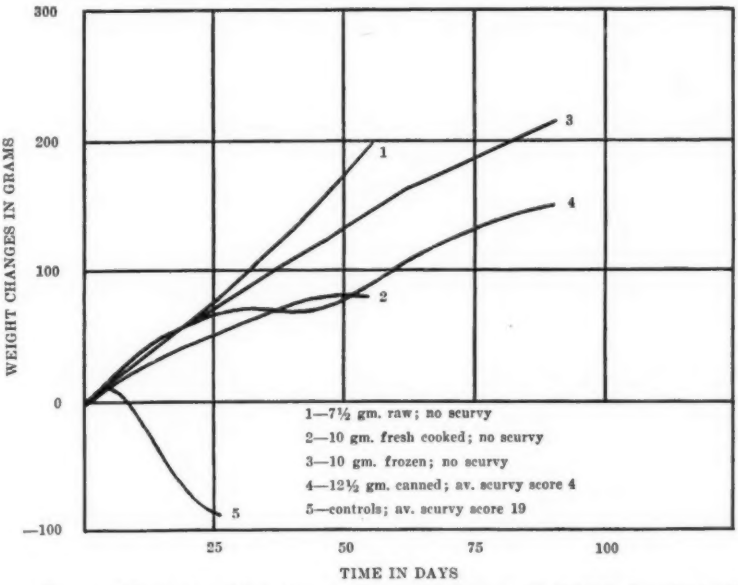

FIG. 1. Effect of cooking, freezing, and canning on the vitamin-C content of Golden Bantam sweet corn.

SUMMARY

Although satisfactory checks were obtained on fresh-raw and fresh-cooked sweet corn between the dye-titration and the animal-bioassay methods, the dye method gave much higher results than did the animal-bioassay method on canned and frozen sweet corn. The oxidation of ascorbic acid while the sweet corn is exposed to air in the feeding cups is a source of error in the assay method.

There is only a small loss in vitamin-C content of sweet corn which has been frozen. Canned sweet corn, with the exception of vacuum-

canned, shows on the average an ascorbic-acid content about 10 per cent less than the average fresh sweet corn; vacuum-canned, whole-kernel sweet corn showed as high vitamin-C content as fresh sweet corn. Sweet corn shipped in cold storage retains its original content of vitamin C to a large extent.

The ascorbic-acid content of various brands of canned sweet corn showed considerable variation but, in general, retained the original content very well. Whole-kernel corn had a higher ascorbic-acid content than cream-style. Hulled corn (lye hominy) was a poor source of vitamin C.

Raw, fresh sweet corn and good-quality, market sweet corn contain about the same amount of ascorbic acid.

Heating sweet corn for eating destroys 7 to 10 per cent of the ascorbic acid.

Sweet corn stored at room temperature and at refrigerator temperatures lost almost no ascorbic acid the first day; only about 20 per cent after three days; and 50 per cent after four days. Negligible loss of vitamin C occurs in storage at —23.3°C.(—10°F.).

Less than five per cent loss of ascorbic acid occurs in frozen sweet corn defrosted for 24 hours.

There is little change in vitamin-C content of sweet corn from the first to the end of the season. There is a general loss during maturation.

CONCLUSIONS

Raw, fresh-cooked (cut or cob), frozen, and whole-grain canned sweet corn are all moderately good sources of vitamin C, i.e., 40 to 60 international units per ounce. Cream-style canned corn is a little lower than whole-kernel.

It is significant that little loss in vitamin C occurs after picking and storage in the husk for a few days. Similarly frozen corn can be defrosted and canned corn kept in the refrigerator for several hours after opening without appreciable loss of vitamin C.

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A PHOTO-ELECTRIC TYNDALL METER[1]

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(Received for publication, January 20, 1937)

Tyndall meters and turbidimeters are particularly adapted to the study of transformations in sols and gels and precipitation, coagulation, and peptization reactions. The instrument here described, which may be useful in certain types of food research, was developed for the purpose of studying the effect of temperature treatments on the light-scattering ability of gelatin sols and gels where a need was felt for a Tyndall meter that would be extremely sensitive to changes in light intensity. It was also desirable to have an instrument which would permit a control of the temperature of the gelatin while determinations were being made. Recently, improved turbidity meters and nephelometers using photo-electric cells have been introduced. These instruments, however, do not have facilities for accurate temperature control of the solution while under observation. While they are portable and no doubt satisfactory for routine laboratory work, their accuracy for certain kinds of research work is questionable.

After some preliminary experimental work the apparatus here described was assembled. It uses as its light-measuring elements two General Electric, PJ - 22 vacuum photo-tubes. This type of tube is particularly useful where a linear response to incident light is required. One photo-tube measures the light transmitted from the solution under observation, while the other photo-tube measures a fraction of the incident light after passing through two Nicol prisms. The photo-tubes are electrically opposed and the current is balanced by rotating the second Nicol prism. A sensitive galvanometer is used as the indicating instrument. The relative intensity of the Tyndall cone is measured in terms of angular degrees in which the Nicol prism must be rotated in order to balance the photo-tubes. By the use of a fundamental physical law these angular degrees are converted into relative light intensities. With this apparatus it is possible to observe the intensity of the Tyndall cone throughout the aging period without disturbing the gelatin-solution cell. Such an arrangement is highly desirable because it prevents moisture condensation when working with temperatures below room temperature.

[1] Contribution No. 269, Massachusetts Agricultural Experiment Station.

DESCRIPTION OF APPARATUS

A schematic diagram of the apparatus and circuit (Fig. 1) shows that the light W is a 1,000-watt, 110-volt, concentrated-filament, projection-type bulb. The heat-insulated light housing has two openings for the transmission of light. Box B_1 is mounted inside of the thermostatically controlled temperature box T and contains photo-tube P_1

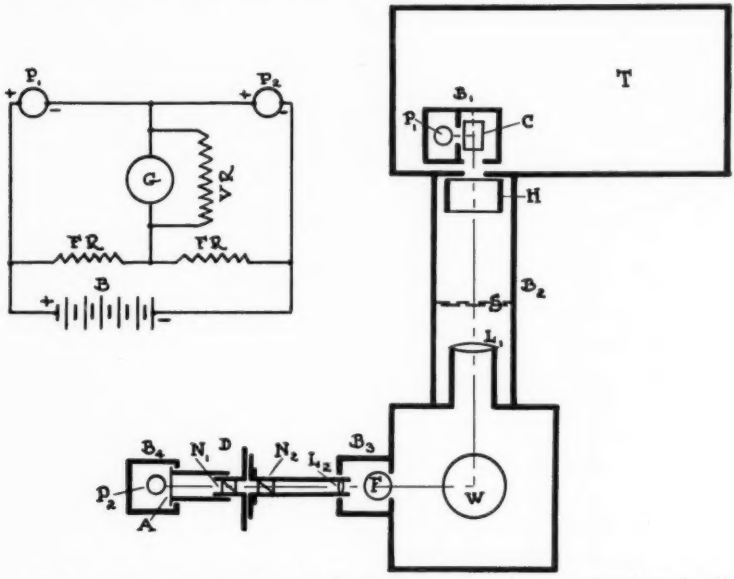

FIG. 1. Diagram of photo-electric Tyndall meter, showing electrical circuit.

G.	Galvanometer
B.	2—45v. batteries
FR.	Fixed resistance, 10,000 ohms
VR.	Variable resistance
P_1, P_2	Photo-electric cells
W.	Light
T.	Thermostatically controlled temperature box
B_1, B_2, B_3, B_4.	Boxes
C.	Glass solution cell
H.	Heat ray filter
S.	Wire screen
L_1, L_2.	Lenses
F.	Globular flask
N_1, N_2.	Nicol prisms
D.	Scale and dial
A.	Ground glass plate

and a glass cell 100 mm. high by 40 mm. square, which holds the solution under observation. A galvanized-iron rectangular, light-proof box B_2 connects the light housing with the temperature box. The lens L_1 renders the light rays parallel before passing through the heat filter H

and through the solution cell C. An opening in the side of the lamp housing permits passage of the comparison beam of light. A 50-c.c. globular flask F filled with distilled water is fastened to the lamp housing in front of the opening. This flask serves as a condensing lens as well as a water cell for removing part of the heat rays. After leaving the flask, the comparison beam passes through the lens L_2 and then through two Nicol polarizing prisms, 12 mm. aperture, and on into box B_4 which contains photo-tube P_2. The ground-glass plate A diffuses the light evenly over the active surface of the photo-tube. The Nicol N_2 is fixed in its position, while Nicol N_1 is movable. The Nicol prisms are adjusted so as to shut out all light when the dial reads 90° and to show maximum brightness when the dial reads zero. This setting of N_2 then allows one to read the angle directly through which it is necessary to rotate the Nicol N_1 in order to equal a particular intensity of Tyndall cone. The square of the cosine of this angle multiplied by 100 gives the "relative Tyndall intensity." Justification for this procedure can be found in various treatises on light.

The photo-tubes were used in a balanced bridge circuit (Fig. 1). A Leeds and Northrup type R galvanometer, having a sensitivity of .003 μ A, is used to determine the null point. A lamp and scale are convenient for measuring the angular deflection of the galvanometer mirror.

OPERATION OF PHOTO-ELECTRIC TYNDALL METER

The glass-solution cell is filled and put in place inside the thermostatically controlled temperature box, and the lamps of the set and the galvanometer are turned on. The Nicol prism N_1 is rotated until the null point is indicated on the galvanometer scale. A variable resistance R, which is of a Leeds and Northrup dial pattern, resistance box, is used as a shunt across the galvanometer in order to obtain the desired deflection. The angle of rotation of the Nicol is read with the aid of a vernier to .05 of a degree. The relation between these angular readings of the Tyndall meter and the value of relative intensity is shown by the cosine squared curve (Fig. 2). It will be noted that near the extremities of the curve the intensity change per angular degree is considerably less than in the middle portion of the curve. Therefore in order to have the angular readings fall within the most sensitive part of the curve the intensity of the incident light is varied by inserting wire screens in the path of light.

This instrument gives an intensity of zero for conductivity water, and the reproducibility of the instrument is readily checked from time to time by inserting in place of the solution cell a right-angle prism, having the hypotenuse surface silvered and one face of the right angle ground. Such a standard will remain constant.

Among the advantages of the Tyndall meter here described are control of temperature of the solution under observation; circumvention of moisture condensation on the solution cell when using temperatures below that of room temperature; a null measurement, indicated by a sensitive galvanometer; a quickly established equilib-

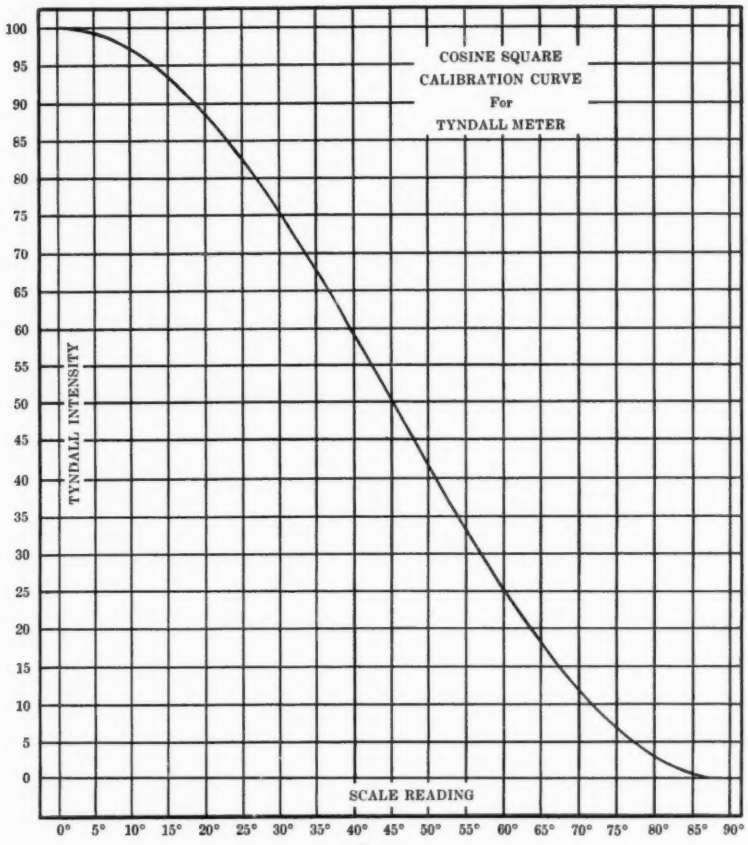

FIG. 2

rium; a negligible drift; and elimination of the personal error which is encountered when comparing two similar intensities of light with the eye. Furthermore, the instrument can be used for relative turbidity measurements by placing the photo-tube P_1 directly back of the solution cell and facing the incident light beam.

The chief disadvantages of this type of Tyndall meter are lack of compactness and portability.

UNDESIRABLE COLOR CHANGE IN FROZEN PEAS STORED AT INSUFFICIENTLY LOW TEMPERATURES[1]

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(Received for publication, February 16, 1937)

Properly scalded peas, frozen and stored at temperatures between —20.6 and —17.8°C.(—5 and 0°F.), have been found by Diehl and Berry (1933) to retain their characteristic bright green color for extended periods of time. On the other hand, collateral samples held at about —6.7°C.(20°F.) gradually lose this color, changing successively from bright green to greenish yellow and greenish brown as the storage period advances. Even peas held at —9.4°C.(15°F.) show a much retarded, although definite and gradual, color change of this nature; while those held at —3.9°C.(25°F.) soon exhibit this phenomenon, even before microbial development begins.

This discoloration seems to occur irrespective of whether the peas are packed in weak brine, in water, or in the dry state. The type of container also has no apparent effect, and the color change occurs in hermetically sealed as well as in non-air-tight containers. Similar color changes occur in spinach and green beans.

Reference to enzymatic or microbiological activity apparently will not explain this color deterioration. In the first case, scalding in boiling water for periods sufficient to inhibit the activity of catalase and peroxidase fails to prevent the change in color. In the second case, although bacteria and "false" yeast may grow in vegetables held at —3.9°C., according to Diehl, Campbell, and Berry (1936), and in artificial media at —8.9°C. (16°F.), as shown by Smart (1935), only molds seem to develop appreciably at —6.7°C. in vegetables, and then only in the presence of air and after long periods of storage, Diehl, Campbell, and Berry. Molds, however, may be excluded as the causative factor, since the color deterioration takes place in the absence of air and of mold growth.

It has been proved that the green color of chlorophyll is dependent upon the presence of magnesium in its molecule and that when magnesium is removed, a yellow to brown decomposition product—phaeophytin—is formed, according to work of Willstätter and Stoll

[1] Food Research Division Contribution No. 319.

(1928). It was therefore considered that the color change in frozen-pack peas may be due to the formation of this substance.

EXPERIMENTAL PROCEDURE

In the experimental work thus suggested the following method was employed for the detection of phaeophytin in discolored frozen-pack peas. Chlorophyll extracts were prepared from scalded Alderman peas stored at —20.6 and —6.7°C.(—5 and 20°F.) for one and one-half years. Twenty grams of the material were ground to a pulp in a mortar with sea sand and a small amount of sodium carbonate. The pulp was transferred to a suction filter and extracted five times with about 40 c.c. of acetone. The filtrate was then made up to 40 c.c. with acetone.

The extract prepared in this manner from the material stored at —20.6°C. exhibited a bright green color, closely comparable to calliste green on Plate IV of Ridgway's color standards, Ridgway (1912). On the other hand, that prepared from material held at —6.7°C. had a color comparable to oil yellow, Plate V of the same standards.

Spectroscopic examination of the above extracts revealed the presence of an absorption band just to the left of Frauenhofer line *E* in the extract prepared from —6.7°C. material. The presence of this band is indicative of phaeophytin, as shown by Willstätter and Stoll. The extract prepared from peas stored at —20.6°C. failed to show the presence of this absorption band.

It is to be noted that magnesium is readily split off from the chlorophyll molecule in *vitro* by weak solutions of acids, such as oxalic, carbonic, boric, and acetic, with the subsequent formation of phaeophytin, according to Willstätter and Stoll (1928) and Jörgensen and Kidd (1917). In *vivo* the decomposition of chlorophyll to phaeophytin by the action of the acids of the cell sap of plants killed by heat and cold has been demonstrated by Wiesner (1876), as cited by Röben (1933).

Apparently the formation of phaeophytin in certain frozen green vegetables stored at —6.7°C. is the result of the action of the acids of the cell sap on chlorophyll. Evidence for the presence in peas of acids capable of altering chlorophyll is supplied by the formation of phaeophytin in acetone extracts of chlorophyll prepared without sodium carbonate as a neutralizing agent. Evidence that these acids are responsible for the color change in peas held at —6.7°C. lies in the fact that scalded peas stored at the same temperature in air-tight containers over ammonia water do not discolor, the acids of the tissues presumably being neutralized by the ammonia absorbed.

CONCLUSIONS

In conclusion, it seems that the factor responsible for the loss of green color in frozen-pack peas stored at insufficiently low temperatures is essentially the slow transformation of chlorophyll into phaeophytin, as a result of the action of the acids of the cell sap. It is presumable, also, that the greenish-brown color of peas which have been subjected to the fermentative activity of the *Lactobacillus* group of bacteria during delay before freezing is also due to the action of lactic acid on the chlorophyll.

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ASCORBIC-ACID CONTENT OF BANANAS AT THREE STAGES DURING RIPENING

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(Received for publication, February 16, 1937)

Results of recent studies of the banana have led to an increasing appreciation of its value as a food in both health and disease. Emphasis is placed on the use of the fully ripe fruit as indicated by a thin, yellow skin overshadowed with brown, and pulp which is soft and has a sweet taste.

The study reported here arose from an interest in the effect that the ripening process might have on the antiscorbutic value of the banana. The possibility of changes in the ascorbic acid during ripening is suggested by several facts: the presence of active enzymes; the conversion of starch to dextrose, levulose, and sucrose; the similarity in structure of ascorbic acid and hexose sugars; and the lability of the acid. Therefore the ascorbic-acid content of bananas taken from the same hand but at three stages during ripening was determined as the first approach to this problem. An effort was made to simulate home conditions of ripening in order that the results would be indicative of the antiscorbutic value of bananas as used in the home.

EXPERIMENTAL PROCEDURE

Ascorbic acid was determined by the 2,6-dichlorophenolindophenol titration method of Bessey and King (1933). Preliminary work indicated that destruction of the ascorbic acid was greatest just following sampling and during the first extraction. This could be prevented by very rapid sampling and by using hot, 18-per cent acetic acid for the first extraction. This was followed by two extractions with eight-per cent acetic acid. Both solutions were made two per cent with respect to metaphosphoric acid as suggested by Mack and Kertesz (1936). Recovery of a known amount of ascorbic acid from lemon juice was complete and this was taken as evidence of applicability of the method under conditions of the experiment.

The management and ripening of the bananas was as follows: Three or four hands[2] of bananas grown in Honduras were delivered

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[2] A bunch of bananas is made up of clusters called "hands," each containing from fifteen to twenty bananas. A bunch usually has from six to nine hands.

from the warehouse on the morning after they had been unloaded from the refrigerator cars. At this stage the fruit was green with deep green tips, the skin was thick, and the pulp very firm, though not so hard as that of raw vegetables. Two or four bananas from each hand were used immediately for the determination of ascorbic acid

TABLE 1

Ascorbic-Acid Content of Bananas from 21 Hands of Fruit at Three Stages of Ripeness

Hands from separate bunches [1]	Ascorbic acid per gram banana (edible portion)		
	Green	Yellow	Ripe
	mg.	*mg.*	*mg.*
A	.054	.057	.057
B	.061	.063	.073
C	.066	.069	.071
D	.....	.....	.073
E	.....	.....	.060
F	.....	.....	.067
G	.069	.073	.082
H	.065	.073	.088
I	.048	.081	.092
J	.060	.053	.076
K	.064	.051	.067
L	.062	.058	.067
M	.063	.049	.064
N	.066	.068	.076
O	.060	.057	.074
P	.056	.057	.075
Q	.056	.057	.073
R	.052	.066	.065
S	.049	.055	.070
T	.049	.055	.066
U	.064	.062	.065

[1] Hands ripened simultaneously are grouped together.

and the results reported for *green* bananas. The hands of fruit were hung in a small room at ordinary temperature. As soon as the fruit had become completely yellow and before it had developed any brown spots (usually at the end of five to seven days), another group was taken for analysis and the results reported for *yellow* bananas. After the next five days the bananas were overshadowed with brown, the skin was very thin, and the pulp was sweet and soft but unblemished.

These were the *ripe* bananas. The results reported here are from analyses completed between November 6 and December 23, 1936.

DISCUSSION OF RESULTS

The results of the chemical analyses are expressed in milligrams of ascorbic acid per gram of banana pulp, giving average figures for each hand at each stage of ripening (Table 1). The ascorbic-acid content of the fruit when the hands were green ranged from .048 to .069 milligram per gram, when yellow from .049 to .081, and when ripe from .057 to .092. The statistical constants for all the data (Table 2) show that the mean ascorbic-acid content is .061, .063, .073 milligram per gram for the green, yellow, and ripe bananas respectively. Similarly the standard deviations are .011, .013, and .011 and the coefficients of variation 18, 20.6, and 14.9 for the respective groups.

TABLE 2

Statistical Constants

Stage of ripeness	Number of bananas	Ascorbic acid per gram banana (edible portion)			Coefficient of variation
		Average	Standard deviation	Probable error	
		mg.	*mg.*	*mg.*	
Green	36	.061	.011	.0071	18.0
Yellow	36	.063	.013	.0084	20.6
Ripe	42	.073	.011	.0071	14.9

Differences in the ascorbic-acid content of the bananas at various stages of ripeness are slight but consistent. The green and yellow fruit differ only within the experimental error of the method. The ripe bananas average .012 milligram per gram more ascorbic acid than the green ones, and the probable error of this difference between the means is .010. Although this is not statistically significant, it is important to note that in no comparison of the same hand was the ascorbic-acid content of the ripe fruit less than that of the green or yellow fruit; it was always equal to or greater than that in the previous stages of ripeness.

These results present favorable evidence for the antiscorbutic value of the ripe banana as opposed to the greener fruit. Indications are that it increases during ripening. That this increase is not due to a parallel dehydration of the fruit is shown by the very slight change in the water content of the pulp during ripening. Average figures for the percentage of water in green, yellow, and ripe pulp are 75.6, 75.9, and 77.4, respectively. Practical significance may be attached to the fact that the greater change in ascorbic-acid content occurs between

the yellow and ripe stages rather than between the green and yellow ones.

Reports in the literature on the milligrams of ascorbic acid per gram of edible portion of ripe bananas range from .021, as determined by Schiaparelli and Buogo (1935); through .15, by Birch, Harris, and Ray (1933); to 1.9, as determined by Everson and Daniels (1936). Bogert (1935) showed that a ripe banana contains 57 International Units per ounce. Since an I.U. is .05 milligram of ascorbic acid, 57 I.U. per ounce would be equivalent to .10 milligram per gram. Comparison of results is difficult since variety, cultivation, storage, speed of ripening, and season all may affect the ascorbic acid in the banana. Moreover, variations in procedures in different laboratories, the extent of sampling, and the number of analyses will influence the results reported.

Biological assay, as given by Rose (1933), places the antiscorbutic value of a 100-gram banana (edible portion) at 17 Sherman units of vitamin C. If the ascorbic-acid equivalent of a Sherman unit is .50 to .75 milligram, the banana would contain between 8.5 and 12.75 milligrams.

From an average of the results of analysis of 42 ripe bananas under the conditions of this study it may be calculated that 100 grams of banana pulp, or the edible portion of a banana of average size, contains 6.2 to 8.4 (7.3 ± 1.1) milligrams of ascorbic acid. This is slightly lower than the biological value cited above or the most recent chemical value of 10 milligrams which is given by the United Fruit Company. These differences are not unreasonable in view of the possible differences in procedures and the developmental status of methods for determination of vitamin C and ascorbic acid.

SUMMARY AND CONCLUSIONS

Ascorbic acid was determined by the indophenol-titration method in bananas from 21 hands at three different stages during ripening.

The results expressed in milligrams of ascorbic acid per gram of banana pulp averaged .061, .063, and .073 for the bananas at the green, yellow, and fully ripe stages respectively. Similarly the coefficients of variation were 18, 20.6, and 14.9. Differences in bananas taken from the same hand at the same stage of ripeness were negligible.

The difference between the mean ascorbic-acid content in milligrams per gram of the green and ripe bananas was $.012 \pm .010$. Although this is not statistically significant it is worthy of note that the ripe bananas taken from the 21 hands were always higher in ascorbic acid than green ones taken earlier from the same hands.

The results reported here indicate a slightly lower ascorbic-acid content of bananas than has been gained from biological assays for

vitamin C. Comparisons with results of chemical assays are difficult since details of management and ripening have not been reported. The present results are somewhat lower than others reported.

Grateful acknowledgment is made to the United Fruit Company and to Mr. J. F. Summers of the Inland Trading Company, Hammond, Indiana, for furnishing and delivering the fruit.

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INFLUENCE OF CERTAIN FRUITS ON FECAL FLORA AND INTESTINAL REACTION IN DIETS OF RATS[1] [2]

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(Received for publication, February 22, 1937)

The suggestion of treating the acute digestive disturbances accompanied by diarrhea, which are so frequent in childhood, with bananas, orange juice, or raw apple, either alone or all together, was made by Fanconi (1930). About this same time Moro (1929) and Heisler (1930) promulgated their raw-apple therapy for diarrhea of young children, based on the old custom among German peasants of using raw apple for this disorder.

A review of the literature indicates that there are at least six factors which may be responsible for the beneficial action of such fruit diets:

1. Tannic acid and astringency, noted by Gebhardt (1935) and McCaslan (1934).
2. Action by encouraging the growth of harmless bacteria and inhibiting the growth of pathogenic organisms, observed by Niszle (1932).
3. Cellulose and pectin content, which swells to give voluminous soft stools, thereby scouring out the intestines and effectively removing bacteria which are basic causes of difficulty, Baumann and Forschner-Boke (1934).
4. Effect on the pH of the intestinal tract which may be such as to discourage the growth of bacteria, Bogert (1935).
5. Carbohydrate content of some fruits, particularly the banana, which may act to bring about a change in the intestinal flora through promoting growth of organisms whose presence creates an unfavorable medium for other types of bacteria.
6. Increased calcium excretion through the mucosa. The increased calcium may inhibit intestinal inflammation and detoxicate amines and phenols by salt formation, Baumann (1932).

[1] Presented before the Central New York State Branch Society of American Bacteriologists, 31st semi-annual meeting in conjunction with the summer meeting of the American Association for the Advancement of Science, Rochester, New York, June 17 and 18, 1936.

[2] Contribution No. 264 of the Massachusetts Agricultural Experiment Station, Amherst, Massachusetts.

In order to study further the apparent beneficial action of fruit diets for intestinal disorders, work has been carried on to note the effect of cranberry, blueberry, and apple diets on the intestinal flora, intestinal putrefaction, and intestinal acidity of the albino rat.

EFFECT OF CRANBERRIES ON INTESTINAL FLORA OF ALBINO RATS

Because of their acidity and astringency it was decided to find out whether or not cranberries, when included in the diet, had any effect on the intestinal flora of albino rats. To date only preliminary work has been done, which consists of feeding experiments with various cranberry diets and noting the amount of acid and gas production in Veillon tubes and the numbers of *Escherichia coli* present in serial dilutions of the feces.

The animals were placed on a basal-colony diet for a two-weeeks' preliminary period, during which time several bacteriological examinations were made of the feces. At the end of this preliminary period varying percentages of dried and raw cranberries were added to the basal ration, and bacteriological studies of the feces were made twice a week for four weeks.

For the bacteriological analysis one-gram samples of fresh feces were placed in sterile dilution bottles containing 99 ml. of water and some broken glass. The bottles were shaken for one-half hour on a shaking machine to break up the feces and get them into solution. From the original fecal suspension, dilutions of 1-10,000, 1-100,000, and 1-1,000,000 were made and one-milliliter portions were inoculated into poured Endo-agar plates and deep Andrade lactose-agar Veillon tubes. The plates and tubes were incubated at 37°C.(98.6°F.) for 48 hours. The diets used and results are shown (Fig. 1 and Tables 1 and 2).

In order to conserve space the detailed experimental data have been omitted except for one series of five rats (Table 1). To date 34 rats have been used on various cranberry diets. From the summarized results (Table 2) it is evident that in general the higher cranberry diets were more effective in bringing about a change in the fecal bacterial flora of albino rats. Of the 34 experimental animals 24, or 71 per cent, showed a decrease in gas production and bacterial activity in the Veillon tubes after being placed on the cranberry diet. Only 22 of the 34 rats had large numbers of *Escherichia coli* in their feces while on the basal diet, but 15, or 68 per cent, of these 22 animals exhibited a decrease in this organism after being placed on the cranberry diets.

EFFECT OF CRANBERRY, APPLE, AND BLUEBERRY DIETS ON INTESTINAL PUTREFACTION

Bergeim (1924) suggested that the degree of reduction of ferric oxide in the digestive tract might serve as a measure of intestinal putrefaction. The fact that ferric oxide when ingested with foods is reduced to the ferrous form and that both forms are readily estimated in the feces, is made the basis of a method for obtaining a quantitative index of reduction processes in the intestine. This method furnishes

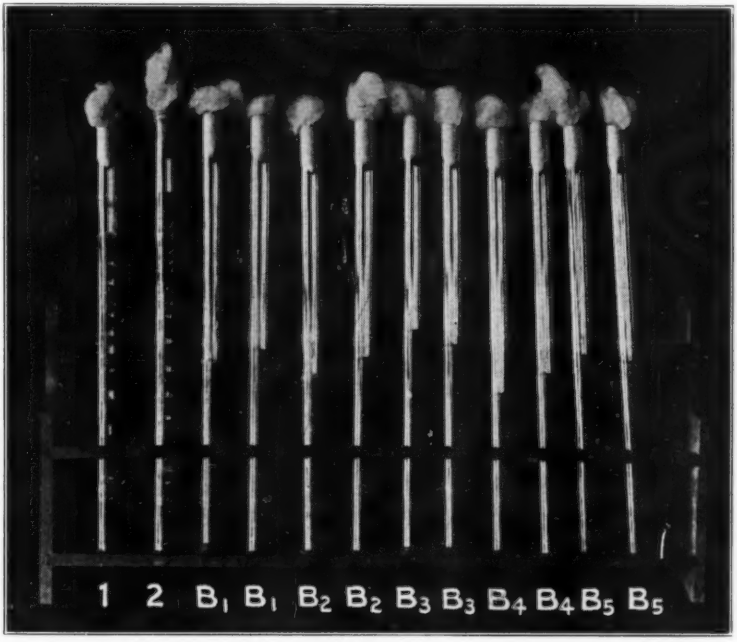

FIG. 1.

Tubes 1 and 2—Gas production in Andrade lactose-agar tubes as produced by test animals on basal diet.

Tubes, Series B—Five animals on basal diet plus 20 per cent dried cranberries for seven days.

a simple index of intestinal putrefaction, the degree of putrefaction varying directly with the degree of reduction.

This experiment was carried out on mature rats. The animals were fed on a basal-colony ration plus one per cent ferric oxide for the first ten days. On the last four days of this preliminary period samples of feces were collected and the degree of reduction of ferric to ferrous oxide was determined. In this way the amount of reduction

on the basal diet for each animal was obtained and could be compared with the degree of intestinal reduction in the same animal when a fruit supplement was added to the basal ration.

TABLE 1

Effect of 20 Per Cent Dried Cranberry Diet on Fecal Gas-Producing and Escherichia coli Bacteria in Albino Rats

Rat	Days on diet	Veillon tubes				*Esch. coli* per gram of feces
		1-10,000		1-100,000		
		Acid	Gas	Acid	Gas	
B_1	3	++++	++++	++++	++++	1,100,000
	5	++++	++++	++++	++++	900,000
	7	++++	++++	++++	++++	1,170,000
	14	++	+	—	—	0
	21	++++	—	++	—	400,000
	38	++++	++++	—	—	1,100,000
B_2	3	++++	++++	++++	++++	3,100,000
	5	++++	++++	++++	++++	1,900,000
	7	++++	++++	++++	++++	6,700,000
	14	++	++	+	—	300,000
	21	+	+—	++	+	0
	38	++++	++++	++++	++++	1,000,000
B_3	3	++++	++++	++++	++++	6,800,000
	5	++++	+—	+++	—	1,900,000
	7	++++	++++	++++	++++	600,000
	14	+	+—	—	—	0
	21	++++	+—	+	—	200,000
	38	++++	++++	—	—	1,100,000
B_4	3	++++	++++	++++	++++	400,000
	5	++++	++++	++++	+	3,300,000
	7	++++	—	+	—	200,000
	14	+—	—	+—	—	100,000
	21	+++	+—	+++	—	0
	38	++++	++++	+++	—	0
B_5	3	++++	++++	++++	++++	3,600,000
	5	++++	++++	++++	++++	3,400,000
	7	++++	+++	++++	+	1,200,000
	14	+—	—	+—	—	100,000
	21	+—	—	+—	—	300,000
	38	++++	++	++++	—	1,800,000

At the end of the preliminary feeding period on the basal ration the animals were given fruit supplements added to the basal ferric-oxide diet. Four different fruit supplements were fed, consisting of 10 per cent dried cranberries, 20 per cent fresh cranberries, 20 per cent fresh McIntosh apples, and 20 per cent fresh cultivated blue-

berries. The degree of reduction was determined over a four-day period after the animals had been on the various fruit diets for seven days.

The results of this experiment (Table 2) give the degree of reduction on the basal diets, after the addition of fruit to the diet, as well as the percentage decrease in reduction due apparently to the fruit. If the Bergeim test for intestinal putrefaction is accurate, it

TABLE 2

Effect of Various Cranberry Diets on Fecal Flora of Albino Rats

Cranberry supplement added to basal diet	Number of rats	Gas production		Numbers of *Escherichia coli*		
		Decrease	No change	Decrease	No change	On basal diet
33⅓% fresh cranberries	15	8	7	5	6	4
5% dried cranberries	4	3	1	1	0	3
10% dried cranberries	5	3	2	2	0	3
20% dried cranberries	10	10	0	7	1	2
Total	34	24	10	15	7	12

is evident (Table 3) that fresh cranberries, blueberries, and apples have a significant effect in decreasing intestinal putrefaction when they are included in the diet.

EFFECT OF APPLE AND CRANBERRY DIETS ON INTESTINAL HYDROGEN-ION CONCENTRATION

Increased intestinal acidity is one of the important factors upon which the value of an aciduric intestinal flora is based. Arnold (1929) found that increased intestinal acidity not only reduces the number of

TABLE 3

Effect of Certain Fruits on Intestinal Putrefaction as Measured by the Reduction of Ferric Oxide

Supplement	Number of rats	Reduction on basal diet	Reduction on basal + fruit diet	Decrease in reduction on fruit diet
		pct.	*pct.*	*pct.*
10% dried cranberries	7	47	43	8
20% fresh cranberries	2	45	33	26
20% McIntosh apples	2	43	33	23
20% fresh blueberries	7	48	32	33

gram-negative intestinal organisms but that it actually causes a reduction of their absorption into other parts of the body. It has been shown that intestinal acidity renders ingested calcium and phosphorus quite soluble, hence they are more completely absorbed into the blood.

Previous workers have shown that the addition of lactose or banana to the diet tends to bring about an increased hydrogen-ion concentra-

tion in the intestinal tract, with its accompanying beneficial change in intestinal flora and increased calcium and phosphorus absorption. Cannon and McNease (1923) state, "The actual hydrogen-ion concentration of the contents of the cecum and colon of the white rat is an important factor in the simplification of the intestinal flora and

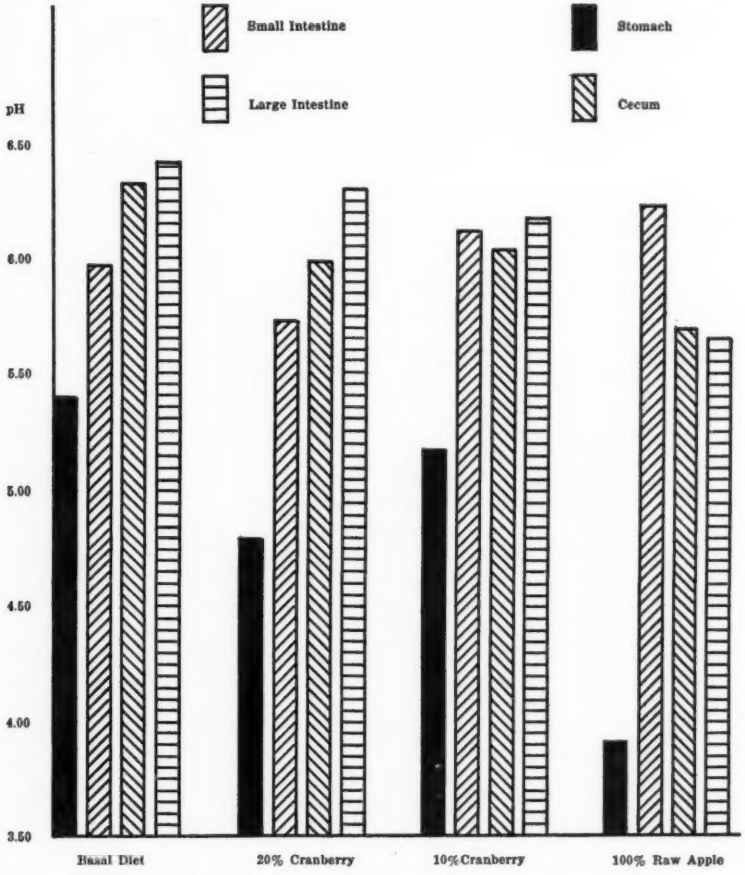

FIG. 2. Effect of fruit diets on the intestinal hydrogen-ion concentration of rats.

the simplification of the intestinal flora varies directly with the pH or acidity; a pH of 7.0 being characteristic of a gas-producing proteolytic type, whereas an increasing acidity is characterized by a decrease in proteolytic types and their replacement by aciduric types mainly dominated by *Lactobacillus acidophilous*."

In view of the increased interest in the use of fruit diets for the treatment of certain intestinal disorders a study has been made of the effect of cranberry and apple diets on the hydrogen-ion concentration, or acidity, of the intestinal contents of albino rats.

For the experimental work 100 rats were placed on a colony diet for a period of three weeks. At the end of this period 42 of the rats were killed and the hydrogen-ion concentration of the contents of the stomach, small intestine, cecum, and large intestine was determined by means of a quinhydrone electrode. The remaining animals were divided into groups and placed on diets consisting of 20 per cent and 10 per cent raw cranberries added to the basal diet, and a straight raw-apple diet. At the end of five days these experimental animals were killed and the pH of the contents of the various intestinal sections was determined. It will be seen (Fig. 2) that these fruit diets produced an increased acidity in the cecum and large intestine, where the maximum bacterial activity is believed to take place. It is evident, therefore, that the inclusion of apples and cranberries in the diet should be of value in promoting an aciduric type of intestinal flora.

SUMMARY

In order to study further the apparent beneficial action of fruit diets for intestinal disorders, work has been carried on to note the effect of cranberry, blueberry, and apple diets on the intestinal flora, intestinal putrefaction, and intestinal acidity of the albino rat. It was found that a 20-per cent cranberry diet is effective in reducing the numbers of fecal gas-producing and *Escherichia coli* bacteria. The Bergeim ferric-oxide reduction test gave evidence that diets of 20 per cent cranberry, blueberry, and apple materially decreased intestinal putrefaction. A study of the hydrogen-ion concentration of the intestinal contents shows that diets of 10 per cent and 20 per cent raw cranberry and of 100 per cent raw apple significantly increased the acidity of the contents of the cecum and large intestine.

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A COMPARISON OF MEDIA AND INCUBATION TEMPERATURES FOR MAKING BACTERIA COUNTS OF SPRAY-PROCESS DRY SKIM MILK

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(Received for publication, February 25, 1937)

INTRODUCTION

That standard nutrient agar and an incubation temperature of 37°C.(98.6°F.) for 48 hours as recommended in "Standard Methods of Milk Analysis" are not the best available means of obtaining the maximum bacteria count in many dairy products has been realized for some time. The quality of milk products has reached a point at present where it is necessary for dairymen and manufacturers to take advantage of the improved laboratory technique made available through various researches, if improvement in the quality of these products is to continue.

The present standard agar and incubation temperature of 37°C. for 48 hours has been in use since 1915. Since that date, research in bacteriological methods has gone a long way. The need for and the advantages of an improved medium and temperature have become more and more apparent.

Media superior to standard agar in a number of ways have been reported by Sherman (1916); Supplee, Whiting, and Downs (1921); Hiscox, Hoy, Lomas, and Mattick (1932); Bowers and Hucker (1935); Safford and Stark (1935), and others.

Supplee, Whiting, and Downs, in testing media at incubation temperatures of 20°C.(68°F.), 30°C.(86°F.), and 37°C.(98.6°F.), found that dextrose agar at 30°C. for five days was superior for developing the maximum bacteria count. Recently Pederson and Yale (1934) reported an incubation temperature of 32°C.(89.6°F.) for 48 hours to result in higher colony counts, minimize the errors owing to variations of incubation temperatures, and give a more consistent percentage of the maximum count than incubation at 37°C. More recent work by Yale and Pederson (1936) has shown that an incubation temperature of 32°C. for 48 hours is fully as desirable with tryptone agar as with standard nutrient agar.

EXPERIMENTAL PROCEDURE

Tryptone agar, proposed by Bowers and Hucker, and an incubation temperature at 32°C. for 48 hours, suggested by Pederson and Yale,

have been studied for some time in this laboratory to determine their effect upon the bacteria counts of spray-process dry skim milk. The tryptone agar proposed by Bowers and Hucker has the following composition:

Tryptone	0.5%
Glucose	0.1%
Fresh skim milk	0.5%
Agar	1.5%

pH 6.7

This medium supports the growth of most bacteria occurring in routine milk samples and also favors the growth of mastitis streptococci, heat resistant and thermophilic bacteria.

The method of analysis was as follows: 10 grams of milk powder were added to 99 c.c. of sterile distilled water from which the neces-

TABLE 1

Classification of 254 Bacteria Counts of Spray-Process Dry Skim Milk Showing the Percentage Increase

Per cent increase	Tryptone agar at 32°C.(89.6°F.) over standard agar at 37°C.(98.6°F.)		Standard agar at 37°C. over tryptone agar at 32°C.	
	Number of samples	Per cent of samples	Number of samples	Per cent of samples
0- 25	45	18.2	19	7.7
25- 50	42	16.9	3	1.2
50- 100	49	19.8	1	0.4
100- 500	63	25.4	0	0
500-1000	11	4.4	0	0
1000-2000	7	2.8	0	0
Over 2000	8			
	225	90.7	23	9.3

Number of samples having the same count..... 6
Total number of samples..... 254

sary dilutions were made. Samples were plated in quadruple on tryptone and standard agar, and duplicate plates were incubated at 37°C. (98.6°F.) and 32°C.(89.6°F.) for 48 hours respectively.

The data herein presented are a summary of the bacterial analysis of 254 samples of spray-process dry skim milk from two plants which were tested over a period of one year.

One hundred fifty-four of a total of 225 counts classified (Table 1) are from 25 to 500 per cent higher on tryptone agar at 32°C. than on standard agar at 37°C. Twenty-six of the 225 are 500 to 2,000 per cent higher.

The averages of the logarithms of the counts on the 254 samples as made by the four procedures, together with the corresponding antilogs, show interesting results (Table 2).

Based on the antilogs of the logarithmic averages the effect of medium, temperature, and medium and temperature combined are given. The advantage of using logarithmic averages is that a single "freakish" count where one sample gives a very much greater difference than the average, has less weight than where arithmetic aver-

TABLE 2

Logarithmic Average of 254 Spray-Process Dry Skim-Milk Counts and Corresponding Antilogs

Standard agar at 37°C.(98.6°F.)	Standard agar at 32°C.(89.6°F.)	Tryptone agar at 37°C.	Tryptone agar at 32°C.
3.87122	3.88632	4.05288	4.15904
7,434.0	7,697.0	11,295.0	14,422.0

ages are used. It is worthy of note (Table 3) that the outstanding effect is in the medium, whereas with other dairy products the big effect is due to the temperature. Tryptone agar at 37°C.(98.6°F.) showed an increase of 51.9 per cent over standard agar at 37°C. Tryptone agar at 32°C.(89.6°F.) gave an increase of 87.4 per cent over standard agar at 32°C., whereas standard agar at 32°C. showed an

TABLE 3

Effect of Medium, Temperature, and Medium and Temperature Combined, Based on the Antilogs of the Logarithmic Averages

	Per cent increase
Effect of medium	
Tryptone agar at 37°C.(98.6°F.) over standard agar at 37°C.	51.9
Tryptone agar at 32°C.(89.6°F.) over standard agar at 32°C.	87.4
Effect of temperature	
Standard agar at 32°C. over standard agar at 37°C.	3.5
Tryptone agar at 32°C. over tryptone agar at 37°C.	27.7
Effect of medium and temperature combined	
Tryptone agar at 32°C. over standard agar at 37°C.	94.0

increase of only 3.5 per cent over standard agar at 37°C. There is a noticeable combined effect of medium and temperature, where both of these factors are varied together, which is greater than the sum of the two when each is varied alone.

An analysis of the effect of the variables on the counts based entirely on arithmetic averages, similar to that used by Kelly (1936), with one exception, was also made (Tables 4a, 4b, and 4c). The per-

TABLE 4a

Distribution of Bacteria Counts of 254 Spray-Process Dry Skim-Milk Samples Based on Arithmetic Averages

Temperature effect on standard agar					
Distribution of counts on standard agar at 37°C.(98.6°F.)	Number of samples	Average count of this group on standard agar at 37°C.	Average count of this group on standard agar at 32°C.(89.6°F.)	Difference in average count	Per cent of increase or decrease
Samples whose 32°C. count on standard agar was above 37°C.					Increase
1- 10,000	91	2,805	3,946	1,141	40.7
10,001- 25,000	19	17,721	22,721	5,000	28.2
25,001-100,000	32	58,609	71,681	13,073	22.3
Over 100,000	15	203,200	245,800	42,600	21.0
Average of all samples above	....	35,131	43,131	8,000	22.8
Total number of samples whose 32°C. count on standard agar was above the 37°C. count on standard agar					157
Per cent of all samples whose 32° C. count on standard agar was above the 37°C. count on standard agar					61.8
Samples whose 32°C. count on standard agar was below 37°C.					Decrease
1- 10,000	49	3,310	2,678	632	23.6
10,001- 25,000	10	15,980	14,070	1,910	13.6
25,001-100,000	17	51,424	46,329	5,095	11.0
Over 100,000	5	149,600	134,200	15,400	11.5
Average of all samples below	....	29,633	33,706	4,073	13.7
Total number of samples whose 32°C. count on standard agar was below the 37°C. count on standard agar					81
Per cent of all samples whose 32°C. count on standard agar was below the 37°C. count on standard agar					31.9
Number of samples having the same count	16	Per cent of samples having the same count			6.3
Total number of samples	254				

TABLE 4b

Distribution of Bacteria Counts of 254 Spray-Process Dry Skim-Milk Samples Based on Arithmetic Averages

Distribution of counts on standard agar at 37°C.	Number of samples	Average count of this group on standard agar at 37°C.	Average count of this group on tryptone agar at 37°C.	Difference in average count	Per cent of increase or decrease
		Media effect at 37°C. (98.6°F.)			
Samples whose count on tryptone agar at 37°C. was above the standard count					Increase
1- 10,000	97	2,895	17,196	14,301	494.0
10,001- 25,000	19	17,526	37,068	19,542	111.5
25,001-100,000	43	57,228	95,409	38,181	66.7
Over 100,000	13	156,384	302,231	145,847	93.3
Average of all samples above	....	29,695	60,488	30,793	103.7
Total number of samples whose count on tryptone agar at 37°C. was above the count on standard agar at 37°C.					172
Per cent of all samples whose count on tryptone agar at 37°C. was above the count on standard agar at 37°C.					67.7
Samples whose count on tryptone agar at 37°C. was below the standard count					Decrease
1- 10,000	46	3,352	2,678	674	25.2
10,001- 25,000	11	15,918	12,800	3,118	24.4
25,001-100,000	6	48,150	44,100	4,050	9.2
Over 100,000	7	251,857	234,643	17,214	7.3
Average of all samples below	....	29,633	49,658	20,025	67.6
Total number of samples whose count on tryptone agar at 37°C. was below the count on standard agar at 37°C.					70
Per cent of all samples whose count on tryptone agar at 37°C. was below the count on standard agar at 37°C.					27.6
Number of samples having the same count	12		Per cent of samples having the same count		4.7
Total number of samples	254				

TABLE 4c

Distribution of Bacteria Counts of 254 Spray-Process Dry Skim-Milk Samples Based on Arithmetic Averages

Media and temperature effect combined

Distribution of counts on standard agar at 37°C.(98.6°F.)	Number of samples	Average count of this group on standard agar at 37°C.	Average count of this group on tryptone agar at 32°C.(89.6°F.)	Difference in average count	Per cent of increase or decrease
Samples whose count on tryptone agar at 32°C. was above the standard count					Increase
1- 10,000	136	2,845	12,084	9,241	325.0
10,001- 25,000	26	16,958	35,069	18,111	106.8
25,001-100,000	46	56,767	98,252	41,485	73.1
Over 100,000	16	203,813	312,188	108,373	53.2
Average of all samples above	....	29,910	53,883	23,973	80.2
Total number of samples whose count on tryptone agar at 32°C. was above the count on standard agar at 37°C.					224
Per cent of all samples whose count on tryptone agar at 32°C. was above the count on standard agar at 37°C.					82.2
Samples whose 32°C. count on tryptone agar was below the standard count					Decrease
1- 10,000	12	4,367	3,792	575	15.2
10,001- 25,000	5	16,960	15,080	1,880	12.5
25,001-100,000	3	46,133	41,733	4,400	10.5
Over 100,000	4	133,750	119,250	14,500	12.2
Average of all samples below	....	29,633	50,430	20,797	70.2
Total number of samples whose count on tryptone agar at 32°C. was below the count on standard agar at 37°C.					24
Per cent of all samples whose count on tryptone agar at 32°C. was below the count on standard agar at 37°C.					9.4
Number of samples having the same count	12		Per cent of samples having the same count		2.4
Total number of samples	254				

centage increases were based on the arithmetic averages of the distribution groups and not on the average percentage difference of the individual counts in the distribution groups.

It is of interest that the majority of the samples showing the greatest percentage increase under the proposed changes are in the low-count range (Tables 4a, 4b, and 4c). This is believed to be in accord with the results reported by Kelly (1936) on other dairy products. Although the percentage increase is high among the low-count samples, the counts *per se* may not necessarily be excessive; e.g., a count of 3,000 bacteria per cubic centimeter on high-quality milk may increase 300 per cent and still be in the low-count bracket, whereas this would not be the case with milk of poor quality.

SUMMARY

Two hundred fifty-four samples of spray-process dry skim milk were plated on standard agar and tryptone agar and incubated at 37°C. (98.6°F.) and 32°C.(89.6°F.) for 48 hours. The bacteria counts were increased by each variation from standard methods. They were increased more by the use of tryptone agar than by lowering the incubation temperature to 32°C. This is contrary to the findings on other dairy products, the counts on other products being increased more by lowering the temperature than by changing the medium as reported by Kelly (1936) and Bradfield (1936). There may be two reasons for this peculiarity of spray-process dry skim milk. First, because of the high temperatures involved in the drying process, the flora of this product must necessarily consist of heat-resistant organisms, the growth of which is favored by the tryptone agar. Second, the desiccation of the milk immediately after the heat treatment eliminates all possibility of growth of organisms at low temperatures; whereas storage of most other dairy products at low temperatures may give opportunity for the growth of organisms, the development of which on the plates would be favored by lowering the incubation temperature.

The greatest percentage increase in the bacteria count of the majority of samples under the proposed changes was in the low-count range.

Tryptone agar gives slightly higher counts at 32°C. than at 37°C., showing a combined effect of medium and temperature.

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SOME OBSERVATIONS ON VITAMIN-C CONTENT OF ORANGES AND LEMONS[1]

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(Received for publication, March 8, 1937)

Oranges and lemons are used so generally in human diet as a source of vitamin C that any specific information concerning the amount of vitamin to be obtained from them is of interest. During a recent study, which involved vitamin-C determinations on large numbers of oranges and lemons, several observations were recorded that give some practical information about these citrus fruits.

ORANGES

The work with oranges continued over a period of six weeks starting early in November, when the California Valencia crop was at the end of its season and the new crop of California navel oranges was just coming on the market. The oranges were cut in halves and the juice was extracted by a "Juicerette." Samples of the freshly extracted juice were at once tested chemically for their vitamin-C (ascorbic-acid) content. The method used was essentially that described by Bessey and King (1933), in which the orange juice was titrated with 2,6-dichlorophenolindophenol and also with standard 0.01 N iodine solution. By following the suggestion of Ahmad (1935), that a drop of glacial acetic acid should be added to the titration flask at the time of titration, any differences owing to slight variations in time seemed to be eliminated, and the end points were sharper.

The average volume of juice that was extracted from Valencia and navel oranges of varied size, and the amount of vitamin C found in these oranges, as determined chemically by the dye and iodine titrations, is shown (Table 1).

The vitamin C (ascorbic acid) per cubic centimeter of orange juice, shown by the dye titration, agrees very closely with the figures given by Daniel, Kennedy, and Munsell (1936) for Valencia and navel oranges from California, when they used the same method.

Since the average volume of juice per orange was determined, the vitamin-C content of a whole orange could be computed. This varies from 20 to 31 mg. by the dye method, or 23 to 32 mg. by the iodine

[1] Approved by the Director of the Montana Agricultural Experiment Station for publication as Journal Paper No. 89, February 20, 1937.

method. One orange would then supply about one-half of the daily requirement of an adult for vitamin C, according to a study reported by Abbasy, Harris, Ray, and Marrack (1935). By comparing the urinary excretion of vitamin C with the intake, they suggest that when the intake of vitamin C is moderately low, about 25 mg. per day, the daily urinary excretion is about 20 mg. This just prevents the earliest

TABLE 1

Vitamin C in Oranges as Determined by Dye and Iodine Titrations

Variety	Size	Av. volume of juice per orange	Av. vitamin C per c.c.		Vitamin C per av. orange	
			Dye titration	Iodine titration	Dye titration	Iodine titration
	No.	*c.c.*	*mg. per c.c.*	*mg. per c.c.*	*mg.*	*mg.*
Valencia choice....	176	52.5	.39	.44	20.5	23.1
Valencia fancy.....	200	50.0	.43	.47	21.5	23.5
Navel fancy.....	200	52.0	.61	.63	31.7	32.8
Navel fancy.....	216	37.5	.62	.68	23.3	25.5

symptoms of vitamin-C deficiency. When the daily intake is more liberal, 50 to 60 mg. (equivalent to the vitamin C found in the juice of about two oranges), the excretion is about 40 mg. This higher rate of excretion indicates a more saturated condition of the blood plasma, which in turn promotes better health, as noted by Greenberg, Rinehart, and Phatak (1936).

LEMONS

During the same period vitamin-C determinations were also made on lemon juice. Sunkist lemons, size 300, were hand-reamed and the

TABLE 2

Vitamin C in Lemons as Determined by Dye and Iodine Titrations

Variety	Size	Av. volume of juice per lemon	Av. vitamin C per c.c.		Vitamin C per av. lemon	
			Dye titration	Iodine titration	Dye titration	Iodine titration
	No.	*c.c.*	*mg. per c.c.*	*mg. per c.c.*	*mg.*	*mg.*
Sunkist fancy.....	300	36.0	.49	.49	17.6	17.6

extracted juice was strained. Immediately the juice was tested by the dye- and iodine-titration methods as described above. The average volume of juice per lemon and its vitamin-C content are listed (Table 2).

These figures represent an average of tests run on 54 lemons, but the individual lemons varied in their vitamin-C content from .26 to .67 mg. per c.c. Lund, Spur, and Fridericia (1934) reported that the vitamin-C potency of lemons ranged from .39 to .56 mg. per c.c. when determined chemically, and from .4 to .6 mg. per c.c., determined bio-

logically. Therefore, there is close agreement in the results from these two laboratories.

It will be noted that when the vitamin-C content of one lemon is computed, it amounts to 17.6 mg., which is slightly less than the smallest amount recorded for one orange (Table 1).

As fresh lemon juice is most frequently consumed in the form of beverages, such as lemonade, fruit punch, etc., where water and sugar are added, it seemed desirable to find out whether such treatment would affect the vitamin C. Tests run on lemon juice, when pure and when made into lemonade, showed no difference in their vitamin-C content. Therefore, a glass (200 c.c.) of freshly made lemonade containing the juice of one lemon would furnish about 18 mg. of vitamin C.

SUMMARY

Chemical tests were run on two varieties of California oranges and on lemons to determine their vitamin-C content.

At the time of year when tested, navel oranges had a higher vitamin-C potency than Valencia oranges.

On an average, one orange contains about 20 to 30 mg. and one lemon, about 18 mg. of vitamin C (ascorbic acid).

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VITAMIN-C CONTENT OF POTATOES PREPARED FOR TABLE USE BY VARIOUS METHODS OF COOKING[1]

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(Received for publication, March 8, 1937)

Potatoes have long been recognized as a valuable source of Vitamin C, and a number of nutrition laboratories have tested their potency when raw and occasionally boiled or baked. Potatoes are prepared for table use in so many ways, however, that it has seemed desirable to determine the vitamin-C content of each type of cooked potato when it is just ready to be served. It would be very difficult to carry out such a plan by means of biological assay, but the chemical method has recently proved sufficiently reliable to be used in this study.

EXPERIMENTAL PROCEDURE

The two varieties of potato most commonly raised in Montana were used in these tests: Netted Gem (Russet Burbank) and Bliss Triumph. They were grown on the irrigated plots of the Montana Experiment Station in Gallatin Valley, were dug about October first, and placed in storage. The vitamin tests were then carried on through the month of November.

Raw Potatoes

Raw potatoes were tested first, so that the vitamin-C content of the cooked potatoes could be compared with that of the original material. Both biological and chemical methods were used.

Biological Method. Guinea pigs, from 250 to 350 grams in weight were placed in individual cages and given a basal diet, recommended by Sherman and modified by Eddy (1929), consisting of rolled oats, 29.5 per cent; wheat bran, 29.5 per cent; butterfat, 9 per cent; cod-liver oil, 1 per cent; common salt (NaCl), 1 per cent; and baked skimmed milk powder, 30 per cent.

Animals used as negative controls received the basal diet only and died of scurvy in about 29 days. Positive control animals received the basal diet plus a daily supplement of six grams of canned tomatoes. Three groups of test animals were given daily supplements of three, five, and seven grams of raw potato, respectively. Gains in weight

[1] Approved by the Director of the Montana Agricultural Experiment Station for publication as Journal Paper No. 90, February 20, 1937.

were recorded and the scurvy score was calculated when the animals were autopsied at the end of the experiment (Table 1).

Chemical Method. Samples of the raw potatoes were subjected to the vitamin-C extraction method described by Rudra (1936). Ten gm. of raw potato were ground in a mortar with 14 gm. of acid-washed sand and 10 c.c. of 20-per cent trichloracetic acid. Ten c.c. of water (freshly boiled and glass-distilled) were added, and the mixture was filtered in a Büchner funnel with suction. The residue

TABLE 1

Vitamin-C Content of Raw and Boiled Potatoes as Determined by Biological and Chemical Methods

Material fed	Number of animals	Gain in weight (56 days)	Scurvy score	Vitamin C	
				Dye titration	Iodine titration
gm.		*gm.*		*mg.*	*mg.*
Netted Gem variety					
3, raw	5	185.0	1.9	0.40	0.52
5, raw	6	175.0	0.2	0.67	0.87
7, raw	6	212.0	0.5	0.93	1.21
Bliss Triumph variety[1]					
3, raw	....	....	....	0.38	0.47
5, raw	5	157.0	0.6	0.63	0.79
7, raw	....	....	....	0.88	1.11
Netted Gem variety					
3, boiled	4	182.7	4.7	0.38	0.50
5, boiled	6	165.0	1.7	0.64	0.84
7, boiled	7	164.2	0.4	0.90	1.18
Bliss Triumph variety[1]					
3, boiled	....	....	....	0.45	0.58
5, boiled	....	....	....	0.75	0.97
7, boiled	....	....	....	1.04	1.36

[1] When the Bliss Triumph potatoes were tested biologically, certain conditions prevented the use of any of the results obtained, with the exception of one feeding level—five grams of raw potato.

was put back into the mortar and ground again with 2.5 c.c. of the trichloracetic acid and 5 c.c. distilled water. This was returned to the filter and the residue washed with 5 c.c. of water. The combined filtrates were then made up to 50 c.c. with glass-distilled water. Ten c.c. aliquots were titrated with 0.01 N iodine and standardized 2,6 dichlorophenolindophenol solutions according to the method described by Bessey and King (1933). The milligrams of vitamin C per gram of raw potato thus determined (Table 2) are used (Table 1) to compare the results of the biological and chemical tests.

Boiled Potatoes

From each variety three potatoes of medium size were selected. They were peeled, weighed, and cooked, one at a time. Each potato was cut crosswise into two parts and dropped into two cups of boiling water, containing one-half teaspoon of salt. Boiling was continued for 35 minutes at a temperature of 95°C.(203°F.). (This lowered boiling temperature is due to the fact that this laboratory is at an

TABLE 2

Vitamin-C Content of Raw and Cooked Potatoes Determined by Chemical Methods

Material tested	Number of tests	Vitamin C	
		Dye titration	Iodine titration
Netted Gem variety			
		mg. per gm.	*mg. per gm.*
Raw	5	.133	.173
Boiled	3	.128	.168
Steamed	3	.141	.176
Pressure-cooked	1	.115	.143
Baked	3	.170	.217
Fried			
American—butter	3	.094	.136
German—butter	3	.118	.143
German—Crisco	3	.137	.176
Mashed	3	.108	.139
Escalloped	3	.094	.125
Bliss Triumph variety			
Raw	3	.126	.158
Boiled	3	.149	.194
Steamed	3	.172	.227
Pressure-cooked	1	.122	.154
Baked	3	.220	.286
Fried			
American—butter	3	.068	.099
German—butter	3	.134	.191
Mashed	3	.146	.205
Escalloped	3	.120	.161

elevation of 4,800 feet above sea level.) When done, the potato was drained and weighed. Immediately a 10-gram sample was taken from the cooked pieces and tested for its vitamin-C content, as rapidly as possible, by the chemical method described above. The average milligrams of vitamin C per gram of boiled potato are shown (Table 2) as well as the change that occurred in the vitamin-C content of the potatoes as a result of boiling (Table 3). It will be noted that Netted Gem potatoes undergo practically no change, but those of the Bliss Triumph variety appear to be more potent after boiling.

TABLE 3

Changes in Vitamin-C Content of Potatoes as Result of Cooking by Various Methods

Material tested	Raw potatoes			Cooked potatoes			Change in vit.-C content as result of cooking
	Total wt. of each potato	Vit. C per gm.	Total vit. C	Total wt. of each potato	Vit. C per gm.	Total vit. C	
Netted Gem variety							
	gm.	*mg.*	*mg.*	*gm.*	*mg.*	*mg.*	*pct.*
Boiled	454.9	.133	60.5	474.1	.128	60.7	0
Steamed	549.2	.133	73.0	570.0	.141	80.4	+10
Pressure-cooked	324.5	.133	43.2	329.2	.115	37.9	—12
Baked A.P.	631.8	.133	84.0	483.2	.170	82.1	— 2
Fried—American							
Potato	426.1	.133	56.7	295.7	.094	27.8	—51
Butter	90.0	.....	.....				
	516.1						
Fried—German							
Potato	721.2	.133	95.9				
Butter	90.0	.....	.....	541.0	.118	63.8	—33
Salt	3.0	.....	.....				
	814.2						
Fried—German							
Potato	497.1	.133	66.1				
Crisco	90.0	.....	.....	290.9	.137	39.9	—40
Salt	3.0	.....	.....				
	590.1						
Mashed							
Potato	1057.1	.133	140.6				
Butter	75.0	.....	.....				
Whole milk	127.2	.018[1]	2.3	1205.7	.108	130.2	— 9
Salt	6.0	.....	.....				
	1265.3		142.9				
Escalloped							
Potato	588.2	.133	78.2				
Butter	48.0	.....	.....				
Flour	7.2	.....	.....	901.4	.094	84.7	0
Whole milk	439.2	.014[1]	6.1				
Salt	3.0	.....	.....				
	1085.6		84.3				
Bliss Triumph variety							
Boiled	630.5	.126	79.4	673.5	.149	100.4	+26
Steamed	599.4	.126	75.5	659.5	.172	113.4	+50
Pressure-cooked	229.5	.126	28.9	231.5	.122	28.2	— 2
Baked A.P.	716.7	.126	90.3	578.6	.220	127.3	+41
Fried—American							
Potato	602.2	.126	75.9	544.8	.068	37.0	—51
Butter	90	.....	.....				
	692.2						
Fried—German							
Potato	772.8	.126	97.4				
Butter	90.0	.....	.....	590.5	.134	79.1	—19
Salt	3.0	.....	.....				
	865.8						
Mashed							
Potato	1190.3	.126	150.0				
Butter	75.0	.....	.....	1254.0	.146	183.1	+21
Whole milk	80.2	.020[1]	1.6				
Salt	6.0	.....	.....				
	1351.5		151.6				
Escalloped							
Potato	618.1	.126	77.9				
Butter	48.0	.....	.....				
Flour	7.2	.....	.....	898.9	.120	107.9	+26
Whole milk	439.2	.017[1]	7.5				
Salt	3.0						
	1115.5		85.4				

[1] Vitamin-C content of milk determined according to Selleg and King, J. Nutr. 11, 599 (1936).

Steamed Potatoes

Three medium-sized potatoes were again selected from each variety and tested separately. Each potato was peeled, weighed, cut in two crosswise, and placed in an ordinary steamer set over a pan of rapidly boiling water. It was steamed for 45 minutes until well done. During this time all moisture that collected in the steamer dripped into the pan below.

The potato was weighed and a 10-gram sample was immediately tested for its vitamin-C content, using the chemical method (Table 2); and the change in the vitamin-C content of the potatoes, as a result of steaming, was determined (Table 3). The potatoes from both varieties gained in weight during this cooking process, especially the Bliss Triumph. At the same time there was an apparent increase in the vitamin C per gram of steamed potato as compared with raw potato. Owing to these two facts the steamed potatoes were exceptionally high in their vitamin-C content.

Pressure-Cooked Potatoes

While the use of a steam-pressure cooker is not recommended for cooking potatoes, in the preparation of a whole meal in the cooker they are often treated in this way. For this reason, pressure-cooked potatoes were included in this series of tests.

A 10-quart National pressure cooker was used with two cups of water in the bottom and the rack raised well above the water. Three medium-sized potatoes from each variety were selected for the test. They were peeled, weighed, cut in halves, and placed in a perforated pan on the rack when the water was boiling rapidly. The cover was securely fastened and the petcock left open until the hiss of steam was sharp. The petcock was then closed and the pressure brought up to 17½ pounds in 5 to 8 minutes. (A pressure of 17½ pounds at this altitude of 4,800 feet is comparable to 15 pounds at sea level.) This pressure was maintained for 12 minutes, then the petcock was opened. The potatoes were weighed and a 10-gram sample was tested at once by the chemical method for its vitamin-C content. It will be noted that pressure-cooking caused some loss in the vitamin potency (Tables 2 and 3).

Baked Potatoes

Separate tests were carried out on three potatoes from each variety. The potato was cleaned, oiled, weighed, and placed in the center of an electric oven with the automatic heat control set at 225°C.(437°F.). It was baked for one hour, then weighed. A 10-gram sample was taken out and tested at once for vitamin C by the chemical method.

The potatoes lost considerable weight during baking (Table 3) but their vitamin-C potency per gram (Table 2) is the highest recorded. When the amount of vitamin C in a baked potato is compared with that in the same potato in a raw state, the Netted Gem showed a slight loss on baking, but the Bliss Triumph, a decided gain (Table 3). These results would seem to contradict those obtained from steaming potatoes. However, in both cooking processes there was an apparent increase in the vitamin-C content of the potatoes, although the weights varied according to the process.

American-Fried Potatoes

Potatoes were boiled by the method previously described, cooled, and placed in an electric refrigerator at 4.4°C.(40°F.) for 24 hours. When each potato was removed, it was weighed and cut in slices about one-eighth inch thick, then placed in an iron frying pan containing 30 grams of hot melted butter. Frying was carried on for 10 minutes, during which time the potatoes were turned with a steel spatula when sufficiently browned. They were then weighed and a 10-gram sample taken for testing by the usual method.

When fried, the slices of boiled Netted Gem potato kept their shape and browned evenly, while the Bliss Triumph slices became mushy, as if water had been added, and browned very little. Netted Gem potato retained a higher concentration of vitamin C per gram than did Bliss Triumph (Table 2), but on the basis of the entire potato, the percentage of loss was the same (Table 3).

German-Fried Potatoes Using Butter

Three potatoes from each variety were tested, one at a time. Each potato was peeled, weighed, sliced raw about one-eighth inch thick, and placed in an iron frying pan containing 30 grams of hot melted butter. One gram of salt was sprinkled over the slices. They were fried at medium heat for 20 minutes, being turned when sufficiently browned. They were then weighed and a 10-gram sample was taken out for testing by the chemical method. The Netted Gem fried potatoes were well browned and very crisp, while the Bliss Triumph were mealy and slightly browned. Netted Gem potatoes lost more vitamin C than did Bliss Triumph under this treatment (Tables 2 and 3).

German-Fried Potatoes Using Crisco

As substitutes for butter are frequently used in the home, one set of tests was carried on with Netted Gem potatoes using Crisco as the fat. The method was identical with that described in the preceding paragraph. The potatoes seemed to brown more than when fried with

butter and had an excellent flavor. The milligrams of vitamin C per gram were greater in the Crisco-fried than in the butter-fried potatoes (Table 2); however, the percentage of loss of vitamin C from the entire mass of fried potatoes was greater in the Crisco-fried (Table 3).

Mashed Potatoes

Three tests were made on each variety, using two potatoes at a time. They were peeled, weighed, and dropped into three cups of boiling water containing two grams of salt. Boiling was continued for 35 minutes at 95°C. (203°F.). They were drained, weighed, and mashed until smooth, with the addition of 25 grams of butter and enough whole milk to give the proper consistency. Netted Gem potatoes required an average of 42.4 grams of milk and Bliss Triumph, 26.7 grams. Just before using, the milk was tested chemically for its vitamin-C content so that could be included in the calculations. The mashed potatoes were weighed and a 10-gram sample was taken for testing. The Bliss Triumph mashed potatoes showed a higher vitamin-C potency per gram than did Netted Gem (Table 2) and appeared to gain in vitamin-C content, while Netted Gem showed a slight loss (Table 3).

Escalloped Potatoes

One dish of escalloped potatoes was prepared for each variety, using four potatoes to a dish. They were peeled, weighed, and sliced about one-eighth inch thick. The other ingredients were weighed out as follows: butter, 48 gm.; flour, 7.2 gm.; and salt, 3 gm. One-fourth of the butter was placed in the bottom of a Pyrex casserole, then one-third of the sliced potato was arranged in a layer and covered with one-third of the flour, salt, and remaining butter. This was repeated twice, then the potatoes were just covered with 439.2 gm. of milk. The uncovered casserole was placed near the center of a heated electric oven, with its automatic control set at 176.7°C. (350°F.), and remained in the oven 70 minutes. After weighing the dish, three 10-gram samples were removed from the center and two ends, respectively, by means of a large cork borer. Each sample was tested for its vitamin-C content. The milligrams of vitamin C per gram of escalloped potato were higher for the Bliss Triumph than for the Netted Gem variety (Table 2). Considering the entire dish of escalloped potatoes, there appeared to be no loss of vitamin C in the case of the Netted Gems, and a considerable gain when the Bliss Triumphs were used (Table 3).

Vitamin C in a Serving of Cooked Potato

In order to compare the results of this study from a practical standpoint, the amount of vitamin found in one serving of cooked

potato has been calculated on the basis of 150 grams, or the weight of one medium-sized boiled potato (Table 4).

DISCUSSION

From the data presented it appears that the two varieties of potatoes used in this study were similar in vitamin-C content when raw. Tested chemically by the dye method, the Netted Gem and Bliss Triumph varieties respectively contained .133 and .126 mg. of vitamin C (ascorbic acid) per gram. Bessey and King (1933) found by dye titration that raw new potatoes contained .17 mg. per gm., while raw old potatoes had .08 mg. per gm., no variety being mentioned. The above results, obtained from potatoes stored about 30 days, fall within the range reported by Bessey and King.

TABLE 4

Vitamin-C Content of One Serving of Cooked Potatoes[1]

Cooking method	Vitamin C in one serving (150 gm.)	
	Netted Gem	Bliss Triumph
	mg.	*mg.*
Boiled	19.2	22.4
Steamed	21.2	25.8
Pressure-cooked	17.3	18.3
Baked	25.5	33.0
Fried		
American—butter	14.1	10.2
German—butter	17.7	20.1
German—Crisco	20.6	....
Mashed	16.2	21.9
Escalloped	14.1	18.0

[1] Comparable to one medium-sized boiled potato.

The results of the biological tests (Table 1) show that feeding a daily dose of five grams of either variety of raw potato gave guinea pigs good protection from scurvy. A minimum protective dose would then appear to be approximately four grams, which would contain about .5 mg. of ascorbic acid, as calculated from the above chemical analyses. This agrees with the fact now accepted by many nutrition workers, that .5 mg. of vitamin C provides a protective feeding level. Thiessen (1936) also reports that 3.5 to 5 gm. of raw Bliss Triumph potato, grown in Wyoming and stored any length of time up to three months, gave protection against scurvy. Woods (1935) found, however, that eight grams of raw Idaho Netted Gem potato seemed necessary to furnish a protective level, which is double the amount reported here. Possibly a difference in soil or some other growth factor may account for a lower vitamin-C potency.

Both biological and chemical tests in this study show that no loss of vitamin C occurred when potatoes were boiled. In the Bliss Triumph variety there even seemed to be a slight increase. Thiessen and Woods, using the same varieties, agree also that boiling causes no loss in vitamin C.

Upon steaming, both varieties showed an apparent increase in vitamin-C potency, greater in the Bliss Triumph (Table 2). The chemical analyses would indicate that 3 to 3.5 gm. of steamed potato would offer protection to guinea pigs. Yanovska (1933) reported that five grams of fresh potato, cooked in steam, furnished a minimum protective dose which is only slightly larger.

Baking also seemed to cause an apparent increase of vitamin C per gram of potato. In fact, the vitamin-C potency of baked potato is the highest recorded (Table 2). However, Woods reported that baked and raw Netted Gem potatoes had the same potency, again showing a decided difference between the results of the Idaho and Montana tests.

Boiled potatoes that were fried in butter (American style) lost about one-half of their original vitamin C (Table 3), while raw potatoes fried in butter (German style) retained more of this factor. In one case where Crisco was used as the fat the fried potatoes contained more vitamin C expressed in milligrams per gram than when butter was used. Yarusova (1936) has reported that margarine frying is much less destructive of vitamin C in potatoes than is ordinary frying, which also indicates that the type of fat used may influence results. Yarusova (1935) further states that a minimum prophylactic dose of fried potatoes of the "Woltmann" variety is at least 15 grams per day, or about twice the amount required of boiled potatoes. This shows a much lower vitamin-C content of fried potatoes than the present work would indicate.

No reports occur in the literature on the vitamin C found in mashed or escalloped potatoes or those prepared in the pressure cooker. However, this study, making use of chemical tests, indicates that no one of these methods is very detrimental to vitamin C. It should be noted that in the preparation of mashed and escalloped potatoes, the addition of milk may dilute the vitamin.

Throughout all the cooking tests the Netted Gem potatoes exhibited a compact, fine-grained structure and tended to retain their shape. On the other hand, the Bliss Triumph potatoes were coarser-grained, with cells readily separated so that they broke up during cooking. However, the Bliss Triumph potatoes generally showed a higher vitamin-C potency after cooking than did the Netted Gem.

It has been noted in several instances that the potatoes, after being cooked by certain methods, showed an apparent increase in the vitamin C (ascorbic acid) as determined by titration. This experimental fact has also been observed in other nutrition laboratories and a controversy has arisen regarding an explanation. Mack (1936) believes that cooking inactivates the oxidizing enzymes so that the total amount of ascorbic acid is obtained. Levy (1936), on the other hand, explains that the apparent increase during cooking is due to a combined form of the ascorbic acid in vegetables.

SUMMARY

Two varieties of potato, Netted Gem (Russet Burbank) and Bliss Triumph, were used in this study to determine the effect of various methods of cooking on their vitamin-C content.

The results of vitamin-C tests, using biological and chemical (dye-titration) methods, were as follows:

a. Approximately four grams of raw potato, from either variety, furnished a minimum protective dose for guinea pigs and the ascorbic-acid content ranged from .126 to .133 mg. per gm.

b. Boiling potatoes 35 minutes at 95°C.(203°F.) caused no loss of vitamin C.

The results of vitamin-C tests, using chemical method only, were as follows:

a. Steaming potatoes for 45 minutes seemed to increase the vitamin-C. content.

b. When potatoes were prepared in a pressure cooker at 17½ pounds pressure for 12 minutes, there was a slight loss of vitamin C.

c. Baking potatoes for one hour at 225°C.(437°F.) caused a definite increase in the amount of vitamin C per gram.

d. Potatoes fried in butter (American style) retained about one-half of the vitamin C contained in the original material.

e. Potatoes fried in butter (German style) lost much less vitamin C than in (d) and the loss was greater in the Netted Gem variety. When Crisco was used as the fat, the vitamin-C content per gram was greater than when potatoes were fried in butter.

f. Mashed potatoes, with milk, butter, and salt added, were comparable to boiled potatoes in vitamin-C potency, although the Netted Gem variety showed a slight loss.

g. Escalloped potatoes retained all of the vitamin C contained in the original material, but owing to the addition of a large amount of milk, the vitamin C per gram of cooked product was diluted.

With one exception Bliss Triumph potatoes, cooked by the various methods, contained more vitamin C per gram than Netted Gem.

The milligrams of vitamin C found in one serving (150 gm.) of potato, cooked by these methods, have been calculated from the chemical analyses.

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ACTION OF MICROÖRGANISMS ON FATS

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(Received for publication, February 27, 1937)

The problem of rancidity continues to occupy the attention of most workers in the field of research on edible fats. The mechanisms of fat spoilage are not generally considered by oil chemists to be due to microbiological action. We believe the conception that the phenomena of rancidity and cleavage are due entirely to such chemical or physical agencies as oxygen, light, positive catalysts (copper, iron, manganese, vanadium, chromium, nickel), Roentgen rays, electromagnetic rays, and moisture should be modified to include enzyme action.

Consideration of the following topics is included here:

a. The importance of lipoclastic activity of microörganisms to the general problem of preservation of edible fats other than butter or margarines as reflected by the literature. We feel that another general review of the evidence taken from the literature can be made without apology.
b. Culture methods to detect lipoclastic actions.
c. Kinds of microbial actions on fats.
d. Diffusion of chromogenic substances into fats (discolorations).
e. Action of bacterial lipases and oxidases on fats stored at —17.8°C. (0°F.) for one year, and hydrolytic cleavage in sweet pickle fats held two months at 2.2 to 3.3°C. (36 to 38°F.).
f. Pure fats as a substrate for growth of microörganisms.

REVIEW OF LITERATURE

Scherer (1795) made the first definite attempt to explain rancidity or spoilage of fats as a chemical change owing to contact with oxygen. Since that time, and especially after Ritsert's (1890) work, rancidification has been considered an oxidative change requiring oxygen which can be accelerated by light. However, when the problem of microbic cleavage of fats in the intestinal tract first came into prominence, Nencki (1886) tested a number of bacteria for lipolytic activities. He observed certain strains of bacteria were lipolytic but recognized that fats are not seriously decomposed by bacteria in the digestion of fats in the intestines. Later Von Sommaruga (1894), Schreiber (1902), and Rubner (1900) found that a large number of

bacteria decompose fats in the presence of atmospheric oxygen and nutrient matter, the action being a hydrolytic cleavage followed by decomposition of glycerol and oxidation of the fatty acids. Orla-Jensen (1902) also observed that bacteria, yeasts, and molds produce lipoclasis in natural fats. He grew actinomyces in sterile butterfat; growth was accompanied by a definite increase in acidity. Eijkman (1901), whose work was confirmed by Söhngen (1911), showed that numerous species of bacteria elaborate lipoclastic enzymes. Söhngen observed many species of bacteria growing well in a substrate where fat was the source of carbon and ammonium chloride the source of nitrogen.

That microbes capable of hydrolyzing fats may be termed "Lipobacteria" was suggested by deKruyff (1906). Wells and Corper (1912) showed the existence of lipase in many species of bacteria killed with toluene. They found Staphylococci, *Pseudomonas pyocyaneus, Bacterium coli, Bacterium dysenteriae, Mycobacterium tuberculosis*—grouped in order of lessening activity—were able to hydrolyze olive oil, triacetin, and ethyl butyrate. Van der Wall (1927) demonstrated lipase in cultures of *Pseudomonas pyocyaneus*, Staphylococci, coliform and dysentery groups of organisms. Kendall, Day, and Walker (1914) have shown lipase in various species of bacteria—*Bacillus proteus* and *Bacillus mesentericus* among them—and Michaelis and Nakahara (1923) extend the list to include *Bacillus prodigiosus*, top yeasts, *Mycobacterium tuberculosis, Staphylococcus aureus* and *albus, Corynebacterium diphtheriae*, dysentery bacilli, coliform group, *Bacterium typhosum*, and *Bacterium paratyphosum B*. Waksman (1929) writes that fats are decomposed chiefly by fungi, with the possible formation of ketones, and by a number of aerobic bacteria including *Staphylococcus aureus, Bacillus prodigiosum, Bacterium pyocyaneum, Bacterium flourescens*, and *Bacterium lipolyticum*, according to Schenker (1921), Derx (1924), Söhngen (1911), deKruyff (1908), and Huss (1908). That microbial lipases show thermolability and thermostability was demonstrated by Söhngen. The lipases of *Bacterium punctatum, Pseudomonas pyocyaneus*, and *Bacillus liquefaciens albus* were found to be more resistant to heat than those of *Bacterium lipolyticum, Bacillus stutzeri, Pseudomonas flourescens, Oidium lactis, Aspergillus niger, Pencillium glaucum*, and *Cladosporium butyri*. Söhngen also believes that he has evidence to show two types of bacterial lipases, alpha and beta, differing in their activity in the presence of acid.

It is claimed by Dubaquie' (1910) that organisms deprived of their usual available food substances will readily utilize fats. The ketonic type of rancidity in coconut oil has been studied by Stokoe

(1928), who finds Penicillia are able to produce methyl, amyl, methyl keptyl, and methyl nonyl ketones from the fat. Taufel, Thaler, and Loweneck (1936) grew *Penicillium glaucum* and *Aspergillus niger* on a variety of substrates and found that formation of methyl ketones takes place within certain limits of (acid) pH values. On palmitic, stearic, and oleic acid substrates mold growth takes place without development of methyl ketone. It was assumed that the unsaturated acids of the oleic acid series behave similarly to the saturated members. Walker (1906) in a thorough study of the cause of rancidity in coconut oil showed that the action of light and air was of relatively little importance in comparison with the changes produced by mold growth. Derx (1925) in a study on oxidation of fats by molds advanced a theory that the formation of methyl ketones by the splitting up of fatty acids proves the oxidation to take place at the beta position of the carbon atom. The fatty acids up to and including myristic acid are adsorbed by the mold mycelium, and thereby hinder the metabolism of the molds. Hence the oxidation of these fatty acids stop at the corresponding beta keto acid, which then splits into CO_2 and methyl ketone.

Davies (1928, 1929, 1930, 1932) has studied ketonic rancidity owing to molds and finds that the liberated fatty acids are fermented to methyl carbinols and ketones which are volatilized. He distinguishes two classes of molds growing on fats, dry and oily. The dry molds are Penicillia, Aspergilli, Monilia, and they possess no apparent lipase. These fungi discolor fats and produce ketonic rancidity. The oily molds liberate oleic acid, giving the fat an oily appearance, liquefaction occurring with prolonged growth. These forms are chiefly *Oidia* and *Oospora* and produce no great discolorations in the fat. The ketonic rancidity of dry mold are reactions with fatty acids mainly up to capric acid (C_{10}), fatty acids from lauric upwards being toxic to them; consequently they produce ketonic rancidity only in those fats containing much of the lower fatty acids up to C_{10} (butterfat and coconut oil). Davies believes that the competitive demand for oxygen for microbial respiration in fatty foods keeps oxygen out of the fat and that oxidative rancidity is started by the acidity formed by microbic agencies previous to the further processing of the fat.

Lewis and Jensen (1928) encountered cultures of oxidizing bacteria which impart tallowy flavors to meat mixtures containing animal fats. However, Rogers, *et al* (1936) write, "A purely chemical change in fats takes place in oxygen and sunlight when oxidation goes on at a slow rate. As a result, the butterfat shows tallowiness in contrast to

the rancidity produced by bacterial action. It is thought that bacteria may also, at times, play a part in the oxidation of fat. It is possible that the bacteria may often be important in the first stages of oxidation and that their protoplasm may act as an acceptor in the process."

Rahn (1905) believes that oxidation prepares the fat so that the bacteria, yeast, and molds will grow well in it. Fats decomposed under anaerobic conditions show, according to Bach and Sierp (1924), a different chemical process. During anaerobiosis, carbon dioxide is split off and the fatty acids are changed into hydrocarbons resulting in formation of products of a lower saponification and higher iodine number than the original fat. Vickery (1936) tested the lipolytic activity of several strains of *Achromobacter* and *Pseudomonas*, and also of *Asporogenous* yeasts, all of which were capable of comparatively vigorous growth on fatty beef tissue held at —18.3°C.(—1°F.). Most of these strains were lipolytic, but only one strain of *Achromobacter* had this power to a slight degree. Jensen and Grettie (1933) succeeded in distinguishing the two main classes of enzymes responsible for rancidity changes—lipases and oxidases. They have evidence to show that microörganisms which produce enzymes of both types are responsible for rapid development both of free fatty acid and of oxidation products. The phenomenon parallels oxidative rancidity of so-called chemical or oxygen-light origin. Decomposition of fats by microbes studied by Horowitz-Wlassowa and Livschitz (1935) indicated that the greater number of bacterial species bring about an initial hydrolysis of the fats, but a few species like *Bacterium lactis aerogenes* may oxidize the fats directly.

Hilditch's (1927) excellent summary of enzyme action should be considered carefully by every technical worker in the field of edible fat production and research. He states, "It is to this class (i.e., enzyme action) that the complications of the rancidity problem are mainly due . . . . *Lipoclastic enzymes*, if the medium is suitable to their development, will tend to the production of free fatty acid. There is some evidence that *enzymes of the peroxidase type* also induce oxidation effects, not only of the same nature as atmospheric oxidation but also in the case of saturated fatty acids, which may be converted by oxidation at the beta carbon atom from the carboxyl group into ketonic acids and ultimately into methyl ketones. . . . Whilst, therefore, the complicated question of the causes contributing to rancidity of edible fats have not yet been thoroughly unraveled, sufficient is known to emphasize the fact that fats prepared for edible purposes and required to be stored for any time prior to use should be kept perfectly sterile and not exposed to contamination by harmful organ-

isms and should also, as far as possible, be excluded from contact with the atmosphere."

CULTURE METHODS TO DETECT FAT CLEAVAGE

A variety of culture media have been used to detect fat-splitting organisms since Nencki's work on lipolysis in 1886. Von Sommaruga (1894) as well as Lieske (1921), Buchanan (1921), Waksman and Daviston (1926), Sayer, Rahn, and Farrand (1908), Turner (1929), Jensen and Grettie (1933), and Collins and Hammer (1934) have employed emulsion fat-agar media containing some indicator substances for acidity, like litmus or brom-phenol-blue; specific indicators for free fatty acid produced by hydrolysis like nile blue sulphate, used by Turner (1929) and Smith (1907); copper sulphate to form blue-green soaps, employed by Carnot and Mauban (1918) and Berry (1933); other metals uniting with fatty acid to form colored soaps; and the diphenylamine and nitric acid test for fatty acid.

Eijkman (1901) inoculated melted agar and then poured it over a layer of beef tallow in a Petri plate. After several days' incubation the fat becomes opaque and fragile in the vicinity of a fat-splitting colony. The fat does not adhere to the glass where this action takes place. In some instances the opaque region is surrounded by a translucent ring and sometimes a clear zone in the tallow underlying the agar. Eyre (1930) uses a sugar-free emulsion-broth filtrate to which he adds a suspension of erythrocytes. Hemolysis of the red blood cells indicates lipase in the original culture. Lipase activity of bacteria has been estimated quantitatively by Michaelis and Nakahara (1923), who used tributyrin as a substrate and measured the activity by the stalagmometric method of Rona and Michaelis (1911).

We have employed these several methods in our laboratory but have found none of them entirely satisfactory. The nile blue-cottonseed oil agar of Turner appeared to approach the ideal medium for the detection and enumeration of lipoclastic or lipase-forming organisms. Cottonseed oil inhibits the growth of many species of microörganisms, however, because of its high active oxygen content. Samples of cottonseed oil examined in this laboratory by Jensen and Grettie (1933) showed active oxygen numbers ranging from five milliequivalents of oxygen per kilogram to 130 milliequivalents. Most vegetable oils, refined and deodorized, tend, upon exposure to air, to possess high oxidizing capacities. It is well known that active oxygen is extremely deleterious to growth of certain species of organisms, the reducing bacteria and fungi being more sensitive to oxidation than the oxidizing flora.

Our modification of emulsion medium which may be incubated either at 20°C.(68°F.) or 37°C.(98.6°F.) is made as follows:

Agar stock { Nutrient agar 100 c.c.
Na_2HPO_4 .5 gm.
pH 7.4

Oil emulsion { 100 c.c. refined coconut oil (or palm oil) 2 gm. gum tragacanth added to hot distilled water 200 c.c. Shake until the globules are approximately 10 μ in diameter. Autoclave the emulsion at 15 pounds for 15 minutes.

Then, as Turner suggests, we add .75 c.c. of the oil emulsion and .75 c.c. of .1-per cent nile blue sulphate aqueous solution to each 5½ c.c. of melted agar. Portions of the well-mixed medium are then cooled to 42°C.(107.6°F.), the inocula in dilutions added, and plates poured in thin layers. Duplicate plates are poured and incubated at 20°C. and 37°C. for four days. The lipase-forming colonies are at first deep blue in color, some colonies taking on a red-copper hue.

The count is made in the manner of the standard water or milk count; i.e., numbers of lipoclastic bacteria per gram of fat from plates showing 30 to 300 colonies.

We then determine the number of microörganisms that incite and aid in oxidative rancidity, together with lipase activity. This is done by using the medium described above without the addition of nile blue sulphate solution. Incubate as described and then test the colonies for their oxidizing activities (oxidase), according to Schultze (1910), Gordon and McLeod (1928), Ellingworth, McLeod, and Gordon (1929), and Jensen and Morton (1931). This is accomplished by pouring a .5-per cent aqueous solution of dimethylparaphenylenediamine-hydrochloride over the surface of the incubated plate and observing the number of colonies that take on a rose-red color. A .4-per cent aqueous solution of tetramethylparaphenylenediamine-hydrochloride is more sensitive and colors the oxidase colonies a deep purple.

Microörganisms producing lipase are characterized by a transparent or translucent zone, and if upon addition of oxidase-detecting dyes the colonies show both lipase and oxidase, one is probably dealing with an oxidative rancidity producing microörganism.

The Eijkman medium mentioned above may be used with good results in detecting microörganisms possessing the dual powers of lipase production and oxidase-peroxidase-peroxide formation. The number of tallow-splitting colonies are counted and then the dye

solutions are poured over the agar and the number of colored colonies that were shown to be lipoclastic are counted.

A typical experiment with samples of fats showing the number of lipase, oxidase, and both lipase and oxidase formers, together with other microörganisms, is shown (Table 1).

It is our opinion that the lipase-oxidase-forming microörganisms are responsible for the more rapid lipolysis and oxidative rancidity encountered in fat. Microörganisms elaborating only lipase alone do not complicate the problem of rancidity as do the dual-acting microbes.

Samples of sterile leaf lard and sterile hydrogenated cottonseed oil shortening were inoculated with lipase formers, oxidase and peroxide formers, and lipase-oxidase cultures.

After holding two weeks at 37°C.(98.6°F.) the two fats, animal and vegetable, were tested by the following methods: Kreis test, organoleptic, Perkins-Vibrans oxygen absorption by Lewis and Perkins (1930), free fatty acid titration, measure of aldehyde with Schiff's reagent, measure of aldehyde with permanganate, and the acetic acid-chloroform-KI-sodium thiosulfate for active oxygen. Results of the bacterial action on these fats are indicated (Table 2).

It is very difficult to prepare the fat or oil to be tested for microörganisms. One gram of the fat to be tested is liquefied in the 41°C. (105.8°F.) water bath and added to 99 c.c. of warm (41°C.) diluent. The diluent employed is a .1-per cent sodium taurocholate or .1-per cent sodium oleate solution. The first dilution is well shaken so that the globules in the emulsion are under 10 μ. Obviously, the smaller the globule the higher the count of microbes obtained.

It is evident that any plating medium with indicators as now developed will not indicate the type of lipoclastic action caused by microbic activity. Nile blue sulphate does stain free fatty acids, but some tests with "Stanolax" mineral oil emulsion substituted for cottonseed oil in the Turner medium led to the belief that the phenomenon of colored colonies may be explained at times from another viewpoint. Under the microscope we observed that bacteria of the inoculum may concentrate at the interfacial trap in the oil-water emulsion medium and affect the dye in some manner, perhaps changing the oxidation reduction potential in the immediate zone of the interface.

KINDS OF MICROBIAL ACTION ON FATS

The media do not indicate surely whether we are dealing with hydrolysis of a given fat, oxidation, oxidative rancidity, "ketonic rancidity," or other types of spoilage.

TABLE 1

Microörganisms in Samples Carried in Laboratory Until Old and Rancid

Mediums	Leaf lard (per gram)					
	Bacteria		Mold		Both enzymes	
	Lip.[1]	Ox.[1]	Lip.	Ox.	Bact.	Mold
Eijkman's original	34,000	.....	150	.....	.....	.....
Lieske's	40,000	.....	180	.....	.....	.....
Turner's cottonseed oil-nile blue	15,000	.....	80	.....	.....	.....
Coconut oil emulsion agar	42,000	20,000	300	40	15,000	35
Palm oil emulsion agar	44,000	15,000	260	50	10,000	40
Eijkman's tallow plate and oxidizing colony detection	34,000	26,000	120	35	20,000	55
Nutrient agar count			40,000			
Reducing bacteria on agar			30,000			
Anaerobes in glucose brain broth			10			
Proteolyzers			30,000			

Mediums	Hydrogenated vegetable oil shortening (per gram)					
	Bacteria		Mold		Both enzymes	
	Lip.	Ox.	Lip.	Ox.	Bact.	Mold
Eijkman's original	90,000	.....	1,200	.....	.....	.....
Lieske's	112,000	.....	900	.....	.....	.....
Turner's cottonseed oil-nile blue	44,000	.....	300	.....	.....	.....
Coconut oil emulsion agar	85,000	30,000	400	60	20,000	43
Palm oil emulsion agar	114,000	28,000	360	54	15,000	45
Eijkman's tallow plate and oxidizing colony detection	150,000	34,000	120	100	66,000	60
Nutrient agar count			160,000			
Reducing bacteria on agar			120,000			
Anaerobes in glucose brain broth			4,000			
Proteolyzers			8,200			

Mediums	Butterfat (per gram)					
	Bacteria		Mold		Both enzymes	
	Lip.	Ox.	Lip.	Ox.	Bact.	Mold
Eijkman's original	360	.....	1,200	.....	.....	.....
Lieske's	600	.....	1,400	.....	.....	.....
Turner's cottonseed oil-nile blue	25	.....	75	.....	.....	.....
Coconut oil emulsion agar	4,000	1,200	1,600	80	1,200	980
Palm oil emulsion agar	6,000	1,100	1,400	92	1,420	1,200
Eijkman's tallow plate and oxidizing colony detection	420	300	980	50	53	22
Nutrient agar count			3,000			
Reducing bacteria on agar			200			
Anaerobes in glucose brain broth			200			
Proteolyzers			2,800			

[1] Lip = lipase; ox. = oxidase.

The inoculation of a leaf lard and a hardened cottonseed oil shortening (Table 2) indicates something of the chemistry involved. We test the inoculated fats by

(a) accelerated rancidity test for critical peroxide value, by Lewis and Perkins (1930) and King, Roschen, and Irwin (1933);

TABLE 2

Action of Lipase-Oxidase Bacteria on Fats

Bacillary culture	Test	Leaf lard [1]	Hardened cottonseed oil shortening [1]
Lipase former No. 3	Organoleptic	—	—
	Kreis	±	±
	Perkins-Vibrans	±	+
	Free fatty acid	++	++
	Aldehyde-Schiffs	—	—
	Aldehyde-permanganate		
	Active oxygen	—	++
Oxidizing No. 48	Organoleptic	± *tallowy*	± *tallowy*
	Kreis	+	+
	Perkins-Vibrans	++	++
	Free fatty acid	—	—
	Aldehyde	+	+
	Active oxygen	++	++
Combined lipase-oxidase No. 14	Organoleptic	++	++
	Kreis	+	+
	Perkins-Vibrans	++	++
	Free fatty acid	++	++
	Aldehyde	++	++
	Active oxygen	++	++
No culture (control after two weeks)	Organoleptic	—	—
	Kreis	—	—
	Perkins-Vibrans	±	±
	Free fatty acid	—	—
	Aldehyde	—	—
	Active oxygen	—	+

[1] ++ = marked; + = present or positive; ± = not marked; — = negative.

(b) for free fatty acid;
(c) aldehyde and ketone tests;
(d) Kreis test, Powick (1923);
(e) organoleptic test.

These tests show (Table 2) that microörganisms may induce

(1) oxidative rancidity (Culture 14);
(2) hydrolysis with high free fatty acid (lipase formers Culture 3);

(3) tallowiness (oxidizers) in beef fat, mutton fat, and leaf lard (Culture 48 and other experiments);

(4) in addition, wherever oxidizing types of microbes exert their influence, flavor changes take place—some changes are "flavor reversions" such as one finds in deodorized oils and fats, and some are "flavor adjuvants," in which condition the flavor is reinforced and also altered in character.

The data do not elucidate any reaction of microbes which may bring out a positive Kreis test. Apparently all oxidizing cultures and some lipase-forming cultures influence the Kreis reaction. Microbial peroxidase from lipase formers of the type which will oxidize nitroso-hemoglobin, nitrosohemochromogens, and hematin to green compounds, Jensen and Urbain (1936), will also, if worked into fat by means of a mixing machine, oxidize the fat (butterfat, coconut butter) during aeration into odoriferous ketones and aldehydes. With peroxidase from *Streptococcus viridans* broth cultures added to leaf lard and then aerated, one does not perceive the characteristic odor of heptaldehyde although tallowiness and "ketonic" odors are present. *Streptococcus viridans* cultures appear not to contain lipase but do produce much peroxidase, Jensen and Morton (1931).

The products formed during all of the enzyme actions, i.e., lipase, oxidizing actions, and both hydrolysis-oxidation (occurring at the same time), are aldehydes, ketones, lactones, oxy and hydroxy acids, and short chain acids, alcohols, CO_2, water, and acids from acetic to nonylic together with their aldehydes, Jamieson (1932) and Hilditch (1927).

It is apparent that rancidity should not be confused with acidity as is so often the case. It appears that some definite terminology should be striven for in designating a type of microbial spoilage. No doubt some kinds of lipoclasis or fat splitting will be difficult to name. A type was encountered in which hematin from heated traces of blood in the lard caused a very sharp uptake of oxygen. This mechanism was shown by Lepper and Martin (1929) to be due to absorption of oxygen through auto-oxidation of unsaturated fatty acids of fats catalyzed by hematin. Lipase of microbial origin speeded the rate of the reaction. Sometimes the microbial, mold, or enzyme actions are superimposed on "chemical" types of rancidity and thus confuse the kind of spoilage. We hold that the microbes to be tested for fat-splitting action should be inoculated directly into a continuous fat rather than into an emulsion medium. Otherwise the end-products of lipoclasis cannot be determined. The emulsion media do, however, show hydrolyzing and oxidizing colonies of bacteria, torulae, and molds.

The end-products of bacterial and mold action in proteins and carbohydrates have been studied extensively, but in the case of fats we are confronted with greater difficulties both in the few varieties of microbes able to grow on fats and the character of the split products. Our knowledge of the bacterial mechanisms and split products from non-fatty substrates is not always well defined after years of research; hence we are not surprised at the present status of the fat spoilage problem.

DIFFUSION OF CHROMOGENIC SUBSTANCES INTO FATS

The most common fat discolorations owing to microörganisms are various shades of pink and yellow-brown. All three types of microörganisms—bacteria, yeast-torula, and molds—have been shown to be causative agents in pigmentation of fats. Very little is known about the chemical configuration of microbic pigments because only in a few instances have these substances been obtained in pure or crystalline condition. It appears that the majority of these pigments are not soluble in water but are soluble in fat solvents, such as ether, alcohol, acetone, chloroform, and pyridine. The pigments of microbic growth are classed with the group of hydrocarbons or oxyhydrocarbons described as luteins, lipochromes, lipoxanthines, chromolipoids, or carotinoids, according to Palmer (1922). The absorption bands of a bacterial pigment separate it into two, three, or four distinct pigments, Lasseur and Girardet (1925) and Reader (1925).

Zopf (1889) described pigments in eight species of microörganisms. The intensely yellow pigment produced by *Bacillus egregium*, when treated with sulphuric acid and nitric acid, changes into a blue compound (lipocyan reaction). The yellow pigment is fat soluble. An alcoholic solution shows absorption bands, one covering the "F" line, the other between "F" and "G." Zopf found the red pigment of *Spaerotilus roseus* to be fat soluble.

The yellow lipochrome of the staphylococci are soluble in fat; the mineral acids when added to the pigment produce a bright yellow color; whereas potassium hydroxide solution when added to the pigment changes it first to orange-red then to red. A drop of sulphuric acid placed on a boiled residue of the lipochrome, which is a reddish-yellow, will give a play of colors—blue-violet to blue-green. The addition of alkali will produce a purple-red or red color.

While studying pigments of aerobic lipophilic yellow micrococci and bacilli diffused and dissolved into fats, Dr. W. Lee Lewis and the writers observed the curious phenomenon of an oxidation-reduction indicator of bacterial origin. The pigment produced by these races of micrococci and bacilli were canary yellow in color and very

fat-soluble. Petri plates were poured with lard and beef tallow. The yellow-producing bacteria were seeded into the fats and the plates incubated at room temperature 22°C.(71.6°F.) for three weeks. The yellow pigment diffused into the fat as the microörganisms grew. When the fat became rancid and began to show peroxide formation, the pigment changed to a greenish shade and within a few days became blue and purple. The microbes had been isolated from a purple-discolored, (fat side) "back-pack" ham.

Treatment of the yellow pigment (dissolved in fat) with hydrogen peroxide produced the characteristic blue color. The blue oxidation compound was then reduced to the original yellow color with sodium hyposulphite.

These purple discolorations of fats had been observed many times before the science of adequate refrigeration was perfected. The purple "stamping ink" discolorations of the fat side of "back-pack" hams years ago was thought to be due to "labor troubles" because no purple colonies of bacteria could be isolated upon culturing the discolored fat. It is said that Rembrandt, the great painter, showed on one of his canvases the interior of a meat shop of his country and faithfully depicted streaks of purple on the fatty tissue of dressed beef carcasses.

The spontaneous microbiological process involved in producing the purple pigment is as follows:

(1) Certain yellow-producing, non-pathogenic cocci and some races of yellow-forming bacilli grow well on solid fats at temperatures ranging from 7.2 to 37.2°C.(45 to 99°F.).

(2) The yellow pigment formed by these bacteria diffuses slowly into the substrate.

(3) When the fat becomes rancid and peroxides appear, or if the peroxides or oxidases elaborated by lipophilic bacteria discussed before in this paper are present, the pigment assumes a greenish color which in several weeks deepens into blue.

Bacterium luteum produces an orange-yellow fat soluble pigment, the color of which is destroyed by acids. *Bacillus flavus* and *Bacillus aurantiacus* (Frankland) form orange-yellow pigments. The latter organism, when isolated from fats, is differentiated from *Bacillus flavus* by its ability to reduce nitrates to nitrite. *Sarcina flava* is identified by its yellow fat-soluble pigment, turning green upon addition of sulphuric acid, and restoration of the yellow color by sodium hydroxide. *Micrococcus rosea* elaborates a red pigment soluble in fat.

This pigment, when treated with sulphuric acid, becomes blue-green and sodium hydroxide restores the red color. Acetic acid destroys the pigment completely.

We have not succeeded in growing *Bacillus prodigiosus* (several varieties) on pure fats. The pigment is thermolabile and the culture gives off the odor of trimethylamine in a nitrogenous-fat medium.

The Chromobacteria studied are not lipophilic but in agar-oil emulsion media produce the many pigments characteristic of this group.

Pink discolorations are fairly common, and operators who find a pink discoloration in their fat products should look to their processing rather than to a suspected contamination with red labels or artificial sources of dyes. Pink discolorations are usually very sensitive to heat but cannot be effaced by filtration or other physical means. These pigments are not poisonous and have no bearing on public health. Some of these pigments are carotinoids and thus are related to vitamin A.

We have encountered yeast, torula, bacilli, micrococci, and molds producing pink pigments in fats both of vegetable and animal origin. Torula (the variety called "pink yeast" by bacteriologists) are with molds the commonest cause of pink fats. The torula are probably related to *Cryptococcus glutinis,* classified by Hansen (1879) as "Saccharomyces." There are many species of red yeasts related to *Cryptococcus glutinis* and most of them grow well in glycerol.

Occasionally some of the normally grey yeasts, like *Saccharomyces ellipsoideus,* become pink if magnesium or phosphates are present in the substrate, according to Kossowicz (1903) and Will (1912). The coloring matter of these yeasts is probably a carotin. We encountered a curious type of pink discoloration of fats owing to a lipophilic torula described as *Saccharomyces pulcherrimus* by Beijerinck (1918). This yeast, or torula, secretes a colorless chromogen which becomes deep red in the presence of iron salts. This organism, when grown on emulsion-agar, produces a cream-colored colony. When a trace of soluble iron is added to the medium a pink color is produced. The iron content of some commercial fats and oils is great enough to activate the chromogen. Beijerinck suggested that the culture might be used to test for soluble iron salts.

Indol- and skatol-producing bacteria growing on a nitrogenous-fat substrate will produce a pink pigment upon the addition of nitrites. This type of discoloration is the result of the interaction between nitrite (containing nitrate as impurity) and indol and skatol produced by such bacteria as the colon bacilli, Clostridia, hemoglobinophilic varieties, torula, etc. The pigment is known as "nitro indol" and "nitroso-indol nitrate."

HYDROLYTIC CLEAVAGE IN STORED FATS

Ham fat and other sweet pickle fats are subjected to the enzymes of many species of salt tolerant and other kinds of microbes. While it is recognized that moisture, brine, and native lipase of tissue and monocyte origin act upon the fat, microbial enzymes play an important role in breaking up pickle fats. The following experiments (Table 3) show the trend of bacterial action on ham fat. It will be observed that in the two tests followed in the laboratory by total aerobic count;

TABLE 3

Lipoclasis in Sweet Pickle Fats Stored for Two Months at 2.2 to 3.3°C. (36 to 38°F.)

(Laboratory test—pickle inoculated with stock cultures)

Age of 15-17-lb. hams, ham fat No. 1	Free fatty acid	Pickle count—aerobic total per gram—Difco North Gelatin Agar	Turner's medium lipase formers	Oxidizers on nitrosohemoglobin agar	"Stability test" peroxide value on milliequivalents per kilogram—20 hours
1. 72 hrs. (green or fresh)	.10	100,000	15,000	7,000	15
2. 5 days	.30	220,000	70,000	100,000	7
3. 15 days	.61	1,200,000	200,000	500,000	4
4. 30 days	.74	1,000,000	300,000	500,000	2
5. 50 days	.88	560,000	400,000	.....	0
6. 60 days	.94	2,000,000	100,000	1,100,000	0
Age of 15-17-lb. hams, ham fat No. 2					
1. 72 hrs.	.09	3,000	100	120	18
2. 5 days	.12	4,600	310	500	10
3. 15 days	.26	60,000	.....	.....	9
4. 30 days	.50	175,000	112,000	1,200	4
5. 50 days	.55	240,000	160,000	8,000	2
6. 65 days	.62	1,220,000	180,000	700,000	Less 1

oxidizers (nitrosohemoglobin test) used by Jensen and Urbain (1936); Turner's nile-blue method for lipase formers; and the chemical determinations of free fatty acid and "stability," there is a trend towards rancidification with growth of microörganisms.

Bacon fat held at —17.8°C.(0°F.) may become rancid after a period ranging from four months to one year. Some of these fats will not show over one milliequivalent of peroxide per kilogram of surface fat after a year of storage. The original number and kinds of bacteria appear to determine this result. Many of the microbial cells are in a "resting stage" during the holding period and their enzymic actions (hydrolysis and oxidation) are not nullified.

Preliminary experiments indicate that such antioxidants as beta-naphthol and gum guaiac tend to deprive some of the lipophilic organisms tested (a black species of the family *Dematiaceae*, a pink torula, and *Aspergillus niger*) of their fat substrate rather than exerting any germicidal or moldicidal action. This may of course be a general phenomenon in the field of bacteriostasis. These microbes grow scantily in the antioxidant treated lard. The black *Dematiaceae* and *Aspergillus* grow in citric acid (antioxidant) treated lard but it is well known that citric acid is readily utilizable by most molds. The gum guaiac treated lard when supporting growth of these fungi does not appear to become rancid although a tallowy odor appears after prolonged incubation at 36.7°C.(98°F.).

DEHYDRATED AND OTHER FATS AS A SUBSTRATE FOR MICROÖRGANISMS

An ordinary commercial lard free from sweet pickle fats was placed in a five-liter flask and heated 148.9°C.(300°F.) under a vacuum for four hours. The lard, after this treatment, showed less than .01 per cent moisture. Some of the original lard was kept as a control to be used as substrate and to be tested for its moisture content. The original lard contained .3 per cent moisture according to the method of Jamieson (1932). The dehydrated lard and the ordinary lard were then poured into large Petri plates and inoculated when cooled to 36.7°C.(98°F.) with

(1) a strain of "lipophilic" gram-positive bacillus *(Genus Bacillus)*,
(2) a strain of lipase-forming yellow chromogenic micrococcus,
(3) a culture of *Aspergillus niger*.

The dry colonies on agar were used as inocula. A set of plates of dry lard inoculated with the three cultures were placed in a calcium dessicator and incubated four weeks at 36.7°C. There was no evidence of growth on these plates, whereas a set of duplicate plates of the ordinary lard with .3 per cent moisture incubated four weeks at 36.7°C. showed growth of each culture.

A sample of sweet-pickle lard treated as above to remove moisture and volatile matter and inoculated with the three cultures showed slight growth of all strains after six weeks' incubation at 36.7°C. The dehydrated sweet-pickle fat contains carbohydrates, nitrogenous substances, nitrates, nitrites, glycerol, etc.

It is a well-known fact that moisture in excess of .35 per cent renders many fats subject to spoilage, and microbiological spoilage is not least among the factors of rancidification under these conditions.

Preparation of a pure lard, free from fatty acid, nitrogenous compounds, etc., is very difficult. Likewise pure fats from any source

are difficult of preparation. There are substances present in fats containing .2 per cent moisture or less which appear to induce the first stages of microbial growth. We recall the experiments of Sperry and Rettger (1915) who demonstrated that the most active anaerobes or aerobes cannot attack native proteins when deprived of enzymes and when protein is the only source of nitrogen. If a trace of peptone was added, growth and proteolysis began.

Commercial fats showing the following stabilities are vulnerable to microbial action, but the impurities which may start growth have not been determined (Table 4).

The so-called autocatalysis of olive oil so often described may perhaps be due to microbic processes. A drop of rancid olive oil placed in a large amount of sweet olive oil often results in rapid spoilage of

TABLE 4
State of Fats Supporting Bacterial Growth

Fat	Peroxide value milliequivalents per kilogram	Hours blown with air for appearance of rancid odor
Killing fat lards	20	10-23
Hydrogenated cottonseed oil	75	30
Cottonseed oil	125	18
Coconut oil	40	?

the whole. Freshly prepared olive oil may contain over 1,000 bacteria per c.c. according to Chiappella (1903) and rancid oils usually contain many bacteria. Pigulewski and Charick (1928) showed that olive oil is very susceptible to the attack of bacteria.

One sample of olive oil taken from a wooden cask showed 10,000 aerobic bacteria per gram when emulsified in North Gelatin Agar-Difco and incubated six days at 20°C.(68°F.). In this connection Roschen and Newton (1934), in a study of mechanisms of auto-oxidation, found that oxidation products in rancid fats including heptaldehyde were inactive as catalysts in promoting rancidification.

Technique and data given here are not sufficiently refined to enter into a specific discussion concerning the chemistry of microbially induced rancidity, but results carry sufficient probability to indicate further studies on this aspect of the rancidity problem.

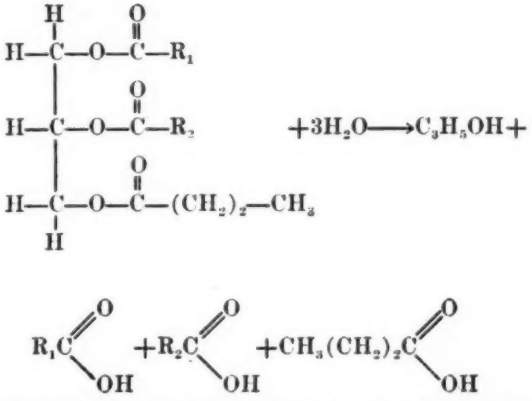
DISCUSSION OF RANCIDIFICATION

Oils and fats from natural sources contain besides fatty glycerides small amounts of other materials, such as high molecular weight alcohols (sterols), hydrocarbons, protein residues and other nitrogenous matter, phosphatides, and carotinoid pigments, which cannot be completely removed by any degree of refinement. Many of these minor

constituents enter into and affect the reactions contributing to spoilage regardless of the source of the oil or fat. Their presence tends to confuse the study of spoilage of oils and fats because they not only may decompose to small amounts of odoriferous substances but may act as catalysts, bacterial substrates, or inhibitors either retarding or promoting reactions contributing to the spoilage of oils and fats.

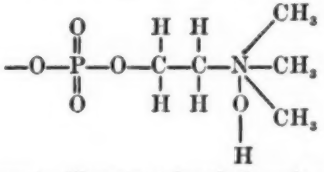
Spoilage of oils and fats, however, appears to involve either one or both of the following types of reactions: hydrolysis and oxidation (or oxidative rancidity).

Hydrolysis may affect either fatty glycerides or certain minor constituents of a fat. Hydrolysis of a fatty glyceride at the ester linkage is shown by the following chemical reaction involving a hypothetical glyceride of butter fat:



The liberation of free butyric acid results in an off-flavored butter which is the only important fat in which hydrolysis of the glyceride would make much difference in flavor or odor.

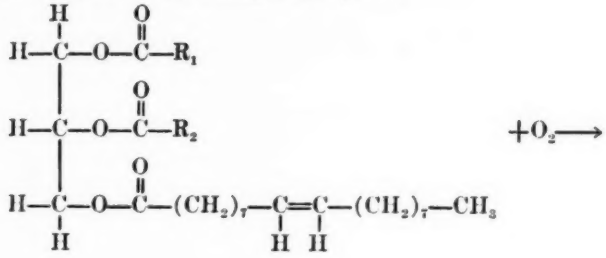
The hydrolysis of lecithin is an example in which hydrolysis of a minor constituent of a fat may cause serious deterioration. Lecithin is a mixed glyceride of two fatty acids and the following radical:

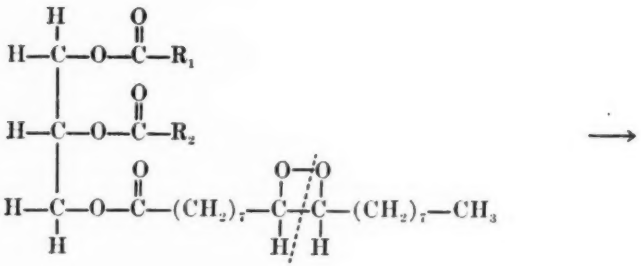


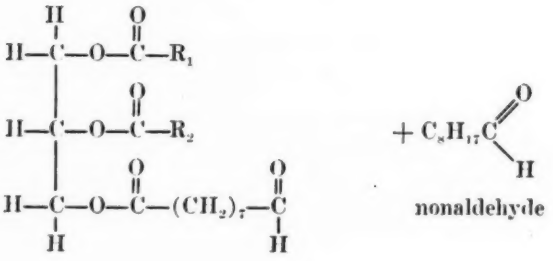
which may hydrolyze to liberate trimethyl amine N $(CH_3)_3$, a compound having a fishy odor. Since lecithin is a common minor constituent in fats and fatty materials, its hydrolysis probably causes fishy flavors and odors often observed in fats. Many species of

microbes are able to effect this hydrolysis. Some of the minor constituents of fats may act as catalysts for the hydrolysis of fats.

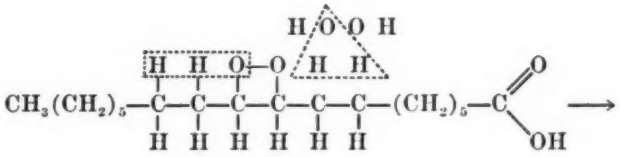
Oxidative changes in oils and fats are the more important causes of spoilage. There are three types of oxidative spoilage recognized in commerce: true oxidative rancidity, ketone rancidity, and flavor reversion. True oxidative rancidity results from the oxidation of the fatty glycerides at the double bonds of unsaturated fat-acid radicals. Oxygen and other gases are dissolved in a fat under ordinary storage conditions. Molecules of the dissolved oxygen occasionally become activated from some exciting cause, often enzymic, and in that condition may unite at the double bonds to form peroxide linkages which are unstable and may break down to form aldehydes, ketones, half aldehydes, and other compounds. The following formulas depict the manner in which these changes take place:

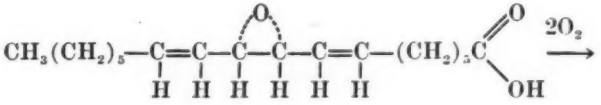


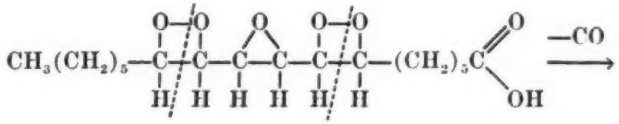


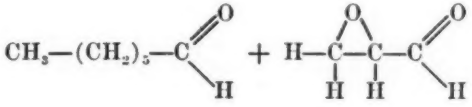


A more complicated reaction is necessary to explain the occurrence of epihydrine aldehyde and heptylic aldehyde in decomposition products of oleic acid. The following reaction was suggested by Powick (1923) to show the oxidation of oleic acid.

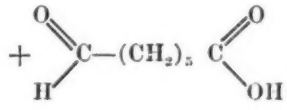








heptaldehyde epihydrine aldehyde



— half aldehyde of pimelenic acid

These compounds are all found in considerable portions in rancid oleic acid. Most oils and fats appear to have small proportions of mixed glycerides of highly unsaturated fatty-acid radicals which oxidize readily to form shorter chain aldehydes and other compounds.

The minor constituents of oils and fats play a very important role in the oxidation of fats in several ways. Some of them may be oxidized

even more readily than the glycerides themselves, resulting in off-flavors. The most important role of the minor constituents in the oxidation of oils and fats is their effects as positive and negative catalysts. Most oils and fats as pressed or extracted from fresh tissue will remain in contact with air or oxygen for a period of time during which there is apparently no oxidation, but at the end of this period oxidation sets in rapidly. This is called the induction period. After oils are highly refined, this induction period is usually shortened or even completely eliminated. If refined oils are treated with any one of a number of definite chemical compounds known as antioxidants or negative catalysts, an induction period is restored in them. Some minor constituent which was present in the crude oil acted as an "antioxidant."

SUMMARY

Methods are indicated for study of hydrolyzing bacteria on emulsified and continuous fat substrates. Certain strains of bacteria producing two kinds of enzymes—lipases and oxidases—appear to be responsible for rapid development both of free fatty acid and of oxidation products. The phenomenon parallels oxidative rancidity of so-called chemical or oxygen-light origin. There is a trend towards rancidification of sweet-pickle fats with growth of lipase-oxidizing bacteria. Bacon fat held at —17.8°C.(0°F.) may become rancid after a period ranging from four months to a year. Numerous determinations of bacteria and stability tests on bacon fats indicate a connection with numbers of bacteria on the original fat and tendency towards rancidity when held at —17.8°C. Moisture-free fats do not support growth of the microörganisms tested, whereas .3 per cent moisture or more in an animal fat aids in promoting growth of the same microbes. Fat-soluble pigments of various microörganisms cause "pink" fats and purple "stamping ink" discolorations by oxidation-reduction mechanisms.

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A REVIEW OF THE BACTERIOLOGY OF FRESH MARINE-FISHERY PRODUCTS

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(Received for publication, March 10, 1937)

Numerous investigators have worked upon various phases of the bacteriology of fish. Some have concerned themselves with determining whether the flesh of fresh-caught fish is sterile. Others have made quantitative studies of the bacteria occurring in fish slime, gills, intestinal tract, and flesh. Several authors have classified the bacteria found on or in sea fish. Attempts have been made to determine whether *Escherichia coli* is a normal inhabitant of the intestinal tract of fish, and the conclusions of the various investigators differ widely. Efforts to prolong the period during which fish may be kept in an edible condition by keeping it in an atmosphere of carbon dioxide or by incorporating a chlorine disinfectant in the ice used to surround the fish are recorded in the literature.

These references are widely scattered and in many instances are found in publications not readily available to those interested in food bacteriology. For this reason a condensed review of the literature has been prepared. Although an effort has been made to cite the majority of articles dealing with the bacteriology of commercial marine fish and fishery products, this is not a bibliography of articles on marine bacteria. Such a bibliography has been published by Bohart (1928). The bacteriology of oysters and shellfish has been reviewed by Hunter and Harrison (1928) and is not included here.

BACTERIA ON THE SURFACE OF FISH

Fish are usually covered with a thin layer of mucus or slime. During life this is constantly sloughing or washing off and may possibly serve to protect the fish against bacterial invasion. Upon death this slime accumulates, and since it is composed mainly of albuminous material and water, it provides an admirable medium for bacterial growth.

Fellers (1926) stated that fish slime is probably responsible for the introduction and growth of bacteria in boats, scows, and on can-

[1] A portion of this work was done while the author was Junior Bacteriologist, U. S. Bureau of Fisheries, and is published with the permission of the Commissioner of Fisheries. Published as Technical Paper No. 261 with approval of the Director, Oregon Agricultural Experiment Station. Contribution of the Department of Fish and Game Management.

nery floors. The bacterial flora which he found in slime was essentially the same as that in the decomposing salmon flesh. Of 75 cultures examined he found the majority to be *Sarcina lactea, Micrococcus varians,* and an acid-forming *Streptococcus.* Members of the coliform group and fluorescent and non-fluorescent bacilli were less numerous but were abundant. The bacterial count of fresh salmon slime was found to be 370 per milliliter. After two hours at 16.7°C.(62°F.) it was 1,950, and after 24 hours it was 3,900,000,000 organisms per milliliter. Hunter (1920b) identified a number of microörganisms from salmon, and he found the same organisms in sea water. Gee (1927a) made isolations from the slime of live haddock and found numerous organisms. Of these about 50 per cent were identified as aerobic spore-forming bacteria of the *Bacillus mesentericus* and *Bacillus vulgatus* types; and of the remaining 50 per cent the more numerous were *Proteus, Pseudomonas,* and *Diplococci.*

Reed and Spence (1929) classified the bacteria recovered from the slimes of 11 haddock. The average distribution of genera was: *Proteus,* 18 per cent; *Achromobacter,* 23 per cent; *Pseudomonas,* 22 per cent; *Flavobacterium,* 8 per cent; *Micrococci,* 4 per cent; *Bacillus,* 24 per cent. Stewart (1932) examined systematically the slime content of 22 haddock. Of the 247 cultures isolated 140 were non-spore-forming bacteria corresponding in general characteristics with the group *Achromobacter.* Fifteen types of *Achromobacter* were recognized of which one, a coccoid bacillus, comprised 70 per cent of the total number of colonies. Forty cultures of micrococcus were described. *Flavobacterium* and *Pseudomonas* occurred often enough to be considered significant. Organisms of the mammalian intestinal type *(Escherichia coli)* were not found, and only four cultures belonging to the genus *Aerobacter* were isolated. Spore-bearing organisms of the aerobic spore-forming group were not encountered in the slime.

Gibbons (1934a) examined the slime from fish caught in the vicinity of Halifax. Sixty-six species of bacteria were isolated from the slime, besides several *Achromobacter* types which could not be classified. Gibbons reported *Achromobacter* and *Micrococcus* types as most frequent. After comparing the organisms isolated from the slime with those isolated from the feces of marine fish he concluded that the flora of both were quite similar. Sanborn (1932) studied the types of organisms occurring on the surface of North Atlantic fish and found a number of organisms which produce slimy or viscous growths on laboratory media. An organism from the surface of halibut which appeared to cause a yellow discoloration on the fish was isolated by Harrison (1929). This was identified as *Pseudomonas fluorescens* and was found to occur in the ice used aboard ship. Bedford (1933)

described eight species of chromogenic bacteria capable of causing a pink or yellow discoloration on the white side of halibut in ten days under the temperature conditions of storage in ice at sea. In a later paper Bedford (1934) discusses the organisms found in sea water and on or in the ocean mud and their relation to the flora of bottom feeding fish. Seventy organisms from North Pacific sea water were grouped in the following genera: *Achromobacter*, 34 per cent; *Flavobacterium*, 30 per cent; *Serratia*, 19 per cent; *Micrococcus*, 16 per cent; and *Rhodococcus*, 1 per cent. Bacterial counts made of the number of organisms on one-gram samples of the skins of 16 haddock varied from 25 to 2,100. These counts were made as soon as possible after the fish were taken from the water and precautions were taken to avoid contamination or multiplication of the numbers present so that the counts probably represent the normal population of bacteria on the fish as near as this can be determined. Lumley, Pique, and Reay (1929) found the number of organisms in a loopful of fresh haddock slime to vary between 100 and 2,000. Flaps of skin about one square inch in area contained bacteria varying in number from 250 to 1,600. Haddock muscle was sterile.

BACTERIA ON THE GILLS

The gills of fish have long been recognized as an important source of spoilage infection, but comparatively little reference was found in the literature in regard to numbers and kind of bacteria growing upon them. Cross (1919) has shown that eviscerated fish, in which the gills remain, putrefied more rapidly than fish from which the gills were removed.

BACTERIA OF INTESTINE AND VISCERA

The number and kind of bacteria in the intestine and viscera of fish is apparently quite variable. Hunter (1920a) and Fellers (1926) found that the digestive tract of migrating salmon was usually sterile. Obst (1919) found the same to be true of sardines. On the other hand, the intestines of feeding fish apparently contain large numbers of bacteria. Browne (1917) examined scup and found members of the *Bacterium coli* and *Bacillus welchii* groups to be present. Stewart (1932) examined the intestinal contents of haddock and found the *Achromobacter* group to be predominant. Spore-bearing organisms were present in small numbers. Harrison (1918) found a bacterium in haddock feces which appeared to be the cause of decay in finnan haddie. Sadler (1918) and Obst isolated from herring feces a number of organisms several of which appeared to be the cause of swells in canned sardines. Sadler, Mounce, and Shanly (1919) found the bac-

teria in sardines to belong chiefly to the *Proteus* and *Bacterium coli-Bacterium aerogenes* genera. Lumley Pique, and Reay found the gut of haddock to contain bacteria varying in number according to the amount of undigested food present. Harrison found the most common organism in the intestines of eight of the twelve haddock examined to be ". . . closely related to B. vulgaris (Hauser)."

Reed and Spence (1929), in their study of the flora of haddock slime and intestine, group the microörganisms into genera. In the intestinal contents of 34 fish they found the following percentage distribution: *Proteus*, 70; *Pseudomonas*, 8.7; *Bacillus*, 5.7; *Coli-Aerogenes*, 4.6; *Flavobacterium*, 5.6; *Achromobacter*, 4.4; miscellaneous, 1. They found a quantitative difference in the frequency of the occurrence of these organisms in the slime and in the intestines. Gibbons (1934a), in his examination of the intestinal and slime flora of 43 fish representing 11 marine species, found that, ". . . taken collectively the flora of the slime is similar to that of the faeces." He includes a valuable key to the genus *Achromobacter*.

Blake (1935) reports the examination of the viscera of 102 fish of the Salmonidae family, 62 from natural waters and 40 from aquaria. Food was absent from 22 of the fish from natural waters. In eight of these both stomach and intestine were bacteria-free, and in seven either the stomach or the intestine was apparently sterile. She states, "the presence of micro-organisms in the alimentary tract depended on food having been taken recently. The stomach and intestine were frequently bacteria-free in fasting fish, and never when food was present (with the exception of bone in one grilse).

"The conclusions drawn were that micro-organisms in the intestines of Salmonidae are derived mainly from food, which is of course influenced by the purity of the water, and there is no persistent commensal bacterial flora in the intestine of these animals."

BACTERIOLOGY OF FISH FLESH

Most investigators have found the flesh of freshly caught fish to be sterile. Hunter and Fellers both reported this to be the case with salmon. According to Anderson (1907) bacterial examination of the muscles of healthy fish indicated that the flesh was usually sterile. Proctor and Nickerson (1935), in examining the flesh of 96 fish, which were frozen with dry ice immediately upon being caught and then examined upon reaching the laboratory, reported all except one sample to be sterile. Harrison, Perry, and Smith (1926) reported the flesh of eight haddock to be sterile.

Contrary to the opinion of the majority of investigators Gee (1930) reported that viable organisms were isolated from the flesh

of five out of 45 fish examined. He isolated from the flesh of live haddock a pleomorphic aerobic spore-forming organism which he identified as *Bacillus mesentericus vulgatus*. Gibbons and Reed (1930) also reported difficulty in consistently obtaining sterile fish tissue. Van Driest (1913) reported that the flesh of haddock and cod examined immediately after death was not sterile. All investigators agree that immediately after death infection occurs. Müller (1903), in one of the first investigations concerning the effect of refrigeration on bacteria, found four species growing on fish flesh at 0°C. (32°F.) Ulrich (1906) determined the bacterial content of fish held at various temperatures. He also examined cooked fish and found that cooking did not ordinarily sterilize the flesh. Brüns (1908) examined 27 varieties of fish and fish products to determine how long after death the flesh at various depths was sterile. After preparing the flesh for the table by frying, baking, or boiling he found it to be sterile. His results disagree markedly with those of Ulrich and later investigators. Browne (1918) investigated the spoilage of fish held in ice. He emphasized the importance of autolytic rather than bacterial changes during the early stages of storage.

Hunter (1920a) studied the bacterial content of the flesh of salmon as it increased on storage. He found an increase of from zero at the time of catching to as high as 155 million per gram after storage for 96 hours at temperatures between 10 and 21.1°C. (50 and 70°F.). Later (1920b, 1922) he identified many of the organisms isolated and stated: "... the bacteria concerned in the decomposition of salmon are those forms the natural habitat of which is the sea water from which the salmon are taken."

Fellers (1926) found: "... the numbers of bacteria increase, as a rule, regularly each 24 hours up to 120 when there is either no increase or else an actual decrease up to 144 hours."—at 10 to 22.2°C. (50 to 72°F.). He agrees with Hunter in that the organisms encountered are those ordinarily found in water. The distribution according to bacterial groups of the 412 cultures isolated from decomposing salmon was: 36.5 per cent aerobic asporogenic non-chromogenic bacilli; 31 per cent cocci; 17.5 per cent aerobic asporogenic chromogenic bacilli; 6.8 per cent yeasts; 4.5 per cent sporogenic aerobes; 2 per cent obligate anaerobes; and 1.7 per cent spirillae. Van Driest found from 100 to 3,200 bacteria per gram in fresh haddock muscle.

In the work of Lumley, Pique, and Reay (1929) counts were obtained of bacteria in haddock stored in ice for varying periods of time. Fish stored for 12 days showed bacterial counts of from 200 to 27,000 per gram. Gibbons (1934c) made a study of the bacteria occurring in haddock fillets which were stored at —18°C. (—0.4°F.)

for periods up to a year. Sixty-eight "varieties" of bacteria were isolated and grouped, as far as possible, according to species. Since it is well established that freezing diminishes the number of organisms but it is unlikely to kill an entire species, the work of Gibbons also indicated the normal flora of unfrozen fish. Harrison, Perry, and Smith (1926) made an extended investigation on the mode of infection of fish flesh, the effect of washing the fish in reducing infection, the effect of storage in increasing the bacterial count, and other phases. They found that the number of bacteria and the rate of increase was larger and more rapid in the flesh near the gills, less numerous and fairly constant at the middle portions of the back, and quite small and slow toward the tail. Of the 68 organisms isolated the predominant groups were *Staphylococcus, Pseudomonas, Flavobacterium, Achromobacter, Escherichia, Bacillus,* and *Serratia.* Although cooking was found to reduce the bacterial count, none of the cooked samples were sterile.

Sanborn (1930) studied the relationship of marine bacteria to the decomposition of fish by determining the proteolytic power of a number of marine bacteria. He concluded: ". . . Marine bacteria are consistently present in and on fresh fish and in smoked and frozen fish. They are active at relatively low temperatures and many of them engage in proteolytic processes in fish muscle. Organisms belonging to the intestinal genera are invariably present in the visceral cavity of split fish and probably come from the alimentary tract. Some definitely putrefactive forms are included in this group which are active in the decomposition of fresh and frozen fish. The removal of contaminating material from the visceral cavity is a wise precaution."

Griffiths and Stansby (1934) attempted to correlate chemical tests with bacterial counts made on the flesh of haddock stored in ice for various periods of time. A sharp rise in values was found to occur after fish had been in ice for from 7 to 11 days. Bacteria counts of 100,000 or more per gram were found to indicate rapid subsequent deterioration, and fish having 1,000,000 or more bacteria per gram were not considered marketable.

Stewart (1935) studied the rate at which decomposition occurred in fresh haddock and in haddock which had been frozen and stored for various lengths of time when both these were kept in melting ice. Measurements of the changes which occurred in volatile nitrogen and in increase in numbers of bacteria were made. As measured by bacterial growth there was no evidence that frozen fish decompose more quickly than fresh fish during 15 days' storage. After this time decomposition is somewhat more rapid in the frozen fish.

Reay (1935) discusses the methods used at the Torrey Research Station, Aberdeen, to estimate the degree of preservation of white fish stored in ice, ice and carbon dioxide, or frozen. He emphasizes the importance of correlating chemical and bacteriological tests with the results of organoleptic examination if a practical method for evaluating the freshness of fish is to be obtained.

OCCURRENCE OF *ESCHERICHIA COLI* AND COLIFORM ORGANISMS IN FISH

Because of the public health significance attached to the occurrence of *Escherichia coli* in water and foods as an indication of potentially dangerous contamination the possibility of its occurrence in the intestinal tract of lower animals and of fish has been investigated.

Amyot (1901) examined 23 fish from Lake Erie and concluded that the colon bacillus is not a normal inhabitant of the intestinal tract of fish. Johnson (1904) examined 67 fish of 15 species and found *Bacterium coli*[1] in 47. Bettencourt and Borges (1908) isolated 29 cultures of colon-like bacteria from the intestines of 17 types of fishes, reptiles, and Amphibia. Only eight of the 29 organisms fermented lactose, and two proved to be typical *Bacterium coli.* Fromme (1910) reported finding *Bacterium coli* in 41 per cent of the fish he examined. Minkewitsch and Trofimuk (1929) examined 232 specimens of fish representing 15 species, including a few of marine origin. Typical mammalian *Bacterium coli* were found in only five cases. Eyre (1904) found *Bacterium coli* in the marine fish he examined. Houston (1905), working in a region remote from sewage contamination, found that only 13 per cent of the fish he examined gave typical *Bacterium coli.* Anderson (1907, 1909) found typical *Bacterium coli* in 25 out of 43 fish taken from the Aberdeen market. He also found coli on the surface of a diseased fish taken from polluted water. Browne (1917) found lactose-fermenting organisms in 73 of 94 scups examined, and identified *Bacterium coli* in 37. Hunter (1920b) and Fellers (1926) found members of the colon-cloacae and aerogenes groups. He stated: "Escherichia coli is not a normal inhabitant of the intestines of salmon." Of the 412 cultures of organisms isolated from decomposing salmon he found eleven to be *Escherichia coli.* Reed and Spence (1929) found coli-aerogenes forms in the intestines but not in the slime of the fish they examined. Gee (1927a) also found a coli-aerogenes type of organism in the course of his examination of the muscle and slime of haddock, but did not regard it as a typical

[1] The nomenclature is quoted as given in the original references. *Bacterium coli* is considered to be the organism now called *Escherichia coli.*

haddock form. Stewart (1932) did not encounter *Escherichia* in her examination of the slime and intestinal flora of the haddock. Gibbons (1934b), in a thorough review of the subject, points out that only one author, Leiter (1929), appeared to have applied Koser and Rettger's (1919) uric-acid and sodium-citrate tests to the intestinal organisms of fish (fresh-water fish only). Gibbons summary is as follows:

"From 110 fish taken in the vicinity of Halifax and from 2 taken at New Haven, 31 strains of bacteria were isolated which produced acid and gas in lactose and 3 which produced only acid in lactose. Ten of these belonged to the genus Aerobacter, eight being Aerobacter aerogenes and two possibly varieties of Aerobacter cloacae. All but one were soil types. One was doubtful.

"Of the 24 classified in the genus Escherichia, eight were faecal mammalian strains and one was doubtful. According to the fermentation reactions, these belonged to the species Escherichia coli, communior, grünthali and Bacterium immobilis. One of these was found in a haddock taken about 3 miles off-shore and in unpolluted waters. All others were found in fish living in contaminated waters (flounder and cunner) or close to shore (mackerel).

"Of 72 off-shore fish, 29 (40.3 per cent) yielded bacteria which produced gas in lactose broth in raw culture. Gram-negative rods were isolated from only 6 (6.9 per cent) of the sugar broth cultures and only one (1.4 per cent) proved to be a typical coli type. Of 40 shore fish, 29 (72.5 per cent) harbored bacteria which formed gas in raw culture. Gram-negative rods were isolated from 24 (60 per cent), of which 8 (20 per cent) were typical coli types." He concluded:

"E. coli, E. communior and A. serogenes are not normal inhabitants of the intestinal tract of marine fishes. Representatives of the genera Escherichia and Aerobacter may be found in marine fishes, but they seldom occur in fish taken some distance off-shore. The faecal forms are particularly rare, except in fish taken near shore or in contaminated waters."

Blake (1935) reports that the bacteria isolated from the intestines of artificially fed fish include a large proportion of types associated with mammalian excreta and decomposition.

Griffiths and Fuller (1936) report the examination of 20 commercial haddock fillets and five eviscerated haddock for members of the coli-aerogenes group. Typical *Escherichia coli* were found on 16 of the fillets and on two of the fish. Lactose-fermenting organisms other than *Escherichia coli* were commonly encountered on both fillets and eviscerated fish. They conclude that many commercial fish and fillets are contaminated through handling.

ATTEMPTS TO CONTROL SPOILAGE OF MARINE FISH

Aside from work showing the advantages to be gained by careful handling and washing of fish, comparatively little has beeen done towards attempting to control or delay spoilage.

Tower (1899) investigated the effect on the keeping quality of fish of different methods of handling, eviscerating, and washing. He found: "a. That putrefaction takes place more rapidly if the viscera are not removed. b. That moisture hastens the process of decay. c. That the free access of air retards putrefaction. d. That drainage of blood retards putrefaction. e. That if the blood and intestines are removed and the fish are suspended by the tail so that the blood is drained from the entire body, the fish will remain sweet for a considerable time without the use of ice."

In an attempt to further delay spoilage by washing the fish thoroughly with disinfectants, .1 per cent salicylic acid in sea water, 10 per cent potassium nitrate, 5 per cent formalin, and 3 per cent boric acid in sea water, were used. Boric acid was the only chemical which delayed spoilage and Tower recommended its use, not as a preservative, but as an agent of cleanliness and to retard the initial stages of decomposition so that the fish may reach the consumer in better condition. Of natural ice Tower says: ". . . the researches of Fraenkel, Bordoin, Uffreduzzi, Prudden, and Heyroth show us that natural ice may contain both putrefactive and pathogenic bacteria. This fact alone should teach us to look with suspicion upon any meat that has been brought in direct contact with ice of unknown origin, especially when the ice is allowed to melt so that the drip soaks into the flesh." Unfortunately Tower's work and recommendations have been long forgotten.

Salmon and LeGall (1936) report that on the basis of differential nitrogen analysis, a prolongation in time of conservation of fish of about 33 per cent results from ozone sterilization of the sea water from which the ice used for packing is made. Browne (1918) concluded that autolytic changes were of more importance during the initial stages of decomposition of fish stored in ice than was putrefaction owing to bacteria. Chen and Fellers (1926) reported the use of hypochlorite solutions for washing fish and they obtained favorable results with ice containing 200 parts per million of available chlorine in inhibiting decomposition of Columbia river smelt (Thaleichthys pacificus). The chlorine caused a yellow discoloration on halibut. Lumley, Pique, and Reay (1929) pointed out the very considerable reduction in bacterial count effected by thoroughly washing the fish with sea water after gutting. Birdseye (1929) used sea water containing one to five parts per million of free chlorine for washing

fish and a nine-per cent chlorinated salt brine for fillets. A reduction of about 50 per cent in the bacterial count was obtained through brine-washing fillets for 20 seconds. Bedford (1932) has shown that immersion for 30 minutes in a 20-per cent salt solution reduced by over 99 per cent the number of bacteria occurring on the surface of halibut. By washing the fish in this concentration of salt the keeping quality was enhanced, bacterial discoloration was avoided, and the washed fish reached the market in a great deal better condition than those unwashed.

Recently attention has been called to the possibilities of using CO_2 gas in the storage of fish to inhibit bacterial growth. Coyne (1932, 1933) has shown that concentrations of from 20 to 100 per cent carbon dioxide inhibit the growth of many organisms, especially *Achromobacter*. He reported that in 20-per cent carbon dioxide growth of organisms responsible for the spoilage of fresh fish is almost completely inhibited. Haines (1933) used concentrations of 10 and 20 per cent carbon dioxide and reported that at 20°C.(68°F.) these concentrations had little action on *Proteus*, but increased the lag period and lengthened by about one-half the generation time of *Pseudomonas* and *Achromobacter*. At 0°C.(32°F.) the generation time of the last two was more than doubled. The maximum number of organisms reached was the same in the presence and absence of CO_2, but the time for attainment of this maximum was approximately doubled at 0°C. by 10-per cent CO_2.

Stansby and Griffiths (1935) tried the effect of carbon dioxide on haddock under conditions closely simulating commercial handling. They state that eviscerated haddock stored in carbon dioxide and under ice until they were unfit for food kept approximately twice as long as those stored under ice in an atmosphere of air. Counts of bacteria in the flesh of fish stored in contact with carbon dioxide were usually much lower than those from control fish. Griffiths (1935) used ice containing ionic silver (Katadyn ice) and ice containing chloromine T in an effort to prolong the length of time fish could be kept fresh. No significant difference was observed between these fish and controls kept similar periods of time in untreated ice.

SUMMARY

From the number of papers referred to it is evident that there exists quite a volume of literature on the bacteriology of fresh marine-fishery products.

Various workers identifying the organisms found on the surface of fish have found *Achromobacter, Micrococci, Pseudomonas, Proteus,* and *Sarcina* to be of frequent occurrence.

The bacteria of the intestine and viscera appear to depend largely on whether the fish is feeding or not feeding, and the type of food being ingested. In a large proportion of the non-feeding fish the intestinal tract has been found to be free of bacteria. From the work reported it appears unlikely that there exist in fish any typical, commensal bacterial flora similar to that which occurs in the intestines of mammals.

Although there is some disagreement as to whether the muscular tissue of living or very recently killed fish is always sterile, most workers agree that infection occurs soon after the fish are caught. The predominating group of organisms found in fish flesh were *Staphylococcus, Pseudomonas, Flavobacterium, Achromobacter, Escherichia, Bacillus* and *Serratia.* Increasing numbers of bacteria in the flesh roughly parallel decreasing quality. Fish containing 1,000,000 or more bacteria per gram of flesh were usually of such poor quality as to be unmarketable.

Escherichia coli is not a normal inhabitant of the intestines or viscera of marine fish. Its occurrence in the intestines or flesh of fish or fillets therefore indicates either that the fish has been taken from polluted water or that contamination has occurred during transportation, handling, or marketing.

Very little work has been done to establish bacteriological standards of quality for fish, and it is desirable that considerably more work be done in order that such standards may be established.

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ORGANIC ACIDS OF THE RIPE BANANA

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(Received for publication, March 20, 1937)

INTRODUCTION

It has been established by Blatherwick (1914) that certain fruits and vegetables are acid-forming foods in spite of the fact that they yield a basic ash. The identity of the organic acids present in the food is the determining factor. If the acids (e.g., malic, citric, etc.) are easily oxidized in the body, the alkaline minerals are left and tend to maintain the alkaline reserve. However, when the acids (e.g., quinic, tartaric, etc.) are oxidized incompletely or with difficulty in the body, the effect may be toxic. If these acids are not neutralized by sufficient alkaline elements in the daily diet, they will be neutralized by the fixed bases of the tissues of the body and a deranged metabolism may result.

The banana has long been known to have an alkaline ash. Sherman and Gettler (1912) placed it among the base-forming foods with a value of 5.6 milliliters of normal acid necessary to neutralize the ash from 100 grams of banana. The value of 6.0 has been established from a number of experiments in this laboratory as the average alkalinity of the ash of Gros Michel bananas, the variety of commerce in the United States. This value is constant for the banana in all stages of ripeness.

The purpose of this study is to identify qualitatively the nonvolatile organic acids of the banana and then to estimate quantitatively the acid or acids present in the fruit at various stages of ripeness.

Several early reports, quoted by Wehmer (1911), indicated that malic acid, unaccompanied by oxalic, acetic, or citric acids, was present in green bananas. The malic acid apparently decreased from .26 per cent in the green fruit to .19 per cent in the partially ripe banana. A malic acid content for ripe bananas of .24 per cent and a titratable acidity of .26 per cent was reported by Nelson (1928). The method employed, however, was that described by Franzen and his associates (1921, 1922, and 1923) in their investigation of a variety of vegetable tissues, and does not lend itself well to quantitative determinations. More recently Hartman and Hillig (1934) in a study of 58 fruits and vegetables found bananas to contain from .37 to .50 per cent malic acid, .15 to .32 per cent citric, no measurable amount

of tartaric acid, and a titratable acidity of .27 to .28 per cent. These investigators used a method developed by themselves, Hartmann and Hillig (1932), for determination of malic acid and standard A.O.A.C. methods for citric and tartaric acids.

EXPERIMENTAL PROCEDURE

Vickery and Pucher (1931) subjected the various qualitative methods of organic acid analysis to a critical study and found that the method employed by Franzen and co-workers (1921, 1922, and 1923) was the only procedure which promised to give trustworthy results. After some modification this method was used by Vickery and Pucher in their investigation of the organic acids in the tobacco plant. Other workers, Nelson and Mottern (1931) and Nelson (1935), have also found this modified Franzen method reliable in qualitative studies on the acids of various fruits and vegetables.

Franzen's procedure as used in the qualitative experiments described here was as follows: 20 kilograms of well-pulped, ripe banana plus an equal amount of water were boiled with continual agitation for 30 minutes. After filtering the mixture through linen, the residue was washed with hot water and finally squeezed nearly dry. The volume of the filtrate was reduced to approximately six liters and then treated with an equal volume of alcohol and filtered to remove most of the pectins. The filtrate was neutralized with the required amount of alkali as determined from the titration of a small aliquot. Lead subacetate was added to complete precipitation and the lead salts were filtered by gentle suction. After thorough washing with dilute alcohol the precipitate was dispersed in water and completely decomposed with sulfuric acid. The lead sulfate was filtered off and washed. The filtrate was freed of any excess sulfuric acid by the careful addition of barium hydroxide, and filtered again. The solution of free acids was evaporated to a small volume and then taken down several times with absolute alcohol to eliminate the last traces of water. The acids were then esterified by refluxing for five hours with absolute alcohol containing 2½ per cent of HCl. The mixture was evaporated on a steam bath and then taken into solution with ether and filtered. The filtrate was washed with dilute NaOH several times until the aqueous layer remained alkaline. The ether solution of the esters was dried over sodium sulfate and then evaporated on a steam bath. The esters were cooled and weighed. A fractional distillation of the esters was carried out under a pressure which varied from 10 to 12 mm. A small Lebel-Henninger distilling column and a Pauly type receiver were utilized. The ester fractions were weighed and then identified by the preparation of the characteristic hydra-

zides. To .7 gram of the ester fraction, dissolved in five milliliters of absolute alcohol, was added one milliliter of a 50-per cent solution of hydrazine hydrate. The hydrazides which crystallized were filtered, washed with absolute alcohol, dried, and their melting-point taken.

TABLE 1

Analysis of the Organic Acids from Ripe Bananas
(Esterified acids from 20 kg. of ripe banana pulp)

Fraction	Weight of fraction	Boiling-point of esters	Melting-point of hydrazide	Remarks
	gm.	*°C. at 10 to 12 mm.*	*°C.*	
Original	28.4	.....	.....	Clear, yellow-colored solution.
1	2.3	125-135	176.0	Characteristic of malic acid.
2	13.6	135-145	176.0-176.5	Characteristic of malic acid.
3	1.3	145-155	176.0	Characteristic of malic acid.
4	2.1	155-165	.....	No hydrazide formed.
5	1.7	165-190	176.0	Characteristic of malic acid.
Residue	3.1	190	.....	Black, gummy residue.
Total	24.1	.....	.....	85% recovery.

It is apparent from the data that malic acid is the principal acidic constituent of ripe bananas (Table 1). To learn how this acid varies in amount during the ripening process, a quantitative study was made.

The method used was that of Hartmann and Hillig (1932), which is essentially an elaboration of Bacon and Dunbar's (1911) procedure. The malic acid is isolated by means of lead acetate and tribasic lead

TABLE 2

Accuracy of the Hartmann-Hillig Malic Acid Method as Used for Bananas

Determination	Number of samples	Sample contained		Malic acid		Recovery
		Banana	Pure malic acid added	Calculated	Determined	
		gm.	*mg.*	*mg.*	*mg.*	*pct.*
1	2	0	50	50.00	51.17	102.34
2	2	0	150	150.00	150.50	100.33
3	5	20	0	.....	40.64	.....
4	5	40	0	.....	81.27	.....
5	3	20	150	190.64	192.64	101.05
6	3	40	50	131.27	133.95	102.04

acetate and then determined by the optical rotation of the uranium-malic acid complex. The results obtained in this laboratory by using Hartmann and Hillig's technique are shown (Tables 2 and 3). In all cases the values presented are averages of two or more determina-

TABLE 3

Malic Acid Content and Titratable Acidity of Bananas During Ripening

Determination	Stage of ripeness		Titratable acidity (ml. N. alkali per 100 gms. banana)	Malic acid		
	Days in ripening room	Appearance of peel		Calculated from titratable acidity	Determined experimentally	Average of determined values
			ml.	*pct.*	*pct.*	*pct.*
1a	0	All green.	3.01	.202	.060	.053
1b	0		2.71	.182	.045	
2a	2	All green.	4.22	.282	.099	.092
2b	2		4.22	.282	.084	
3a	4	Green, traces of yellow.	4.56	.306	.384	.373
3b	4		4.48	.300	.361	
4a	7	More yellow than green.	5.48	.367	.361	.358
4b	7		5.36	.359	.354	
5a	9	Yellow, green tip.	5.10	.342	.335	.352
5b	9		5.14	.395	.369	
6a	11	Full yellow color.	4.88	.327	.312	.333
6b	11		4.76	.319	.354	
7a	14	Yellow flecked with brown.	4.82	.323	.305	.314
7b	14		4.82	.323	.322	
8a	16	Black-colored skin indicating dead ripeness of fruit.	4.56	.306	.305	.301
8b	16		4.48	.300	.297	

tions. The procedure itself, while extremely arbitrary and time-consuming, is fairly accurate and quite easily applied.

Two bunches of bananas (Gros Michel from Costa Rica) were set aside for this experiment. One bunch was designated "a" and the other "b." The fruit was ripened normally in accordance with standard commercial practice. Every two or three days samples were taken and the malic acid content determined. Fingers from each bunch were well pulped separately, and a sample in duplicate was weighed out from each.

It might seem that an acid other than malic was indicated by the behavior of Fraction 4 (Table 1). The esters in this fraction gave no hydrazide although smaller fractions boiling directly above and below readily yielded crystalline hydrazide of malic acid. After saponifying a portion of Fraction 4, however, an acid was separated and recrystallized which gave a mixed melting-point with purified l-malic acid identical with that of the l-malic acid itself. It is not known why the diethyl malate (155°-165°) did not form a hydrazide.

Previous to the qualitative estimation, several quantitative determinations for oxalic acid had been made on banana pulp with negative results. The method of Arbenz (1917) for oxalic acid in plant material was used and in no case indicated a measurable amount of this acid in bananas.

All the qualitative data seem to contribute to the conclusion that malic acid is the only nonvolatile organic acid present in the ripe banana. This bears out Bigelow and Dunbar's (1917) generalization that bananas probably contain only malic acid, and is in contradiction to Hartmann and Hillig (1934), who found citric as well as malic acid in bananas.

The results of the malic acid analyses during ripening are shown by a solid line (Fig. 1). The values used are the averages of the figures for bunches "a" and "b" (Table 3). In the same table titratable acidity during ripening is expressed as percentage of malic acid, and these values vary as shown by the broken line (Fig. 1). A typical respiration rate curve is also included in the chart as a matter of interest.

The variation in malic acid content of bananas corresponds to some extent with the respiratory rate of the fruit under the same conditions. There is a great initial increase which reaches a peak on the fourth or fifth day, at which time a yellow tinge is just beginning to appear in the peel, and then gradually decreases. The acid accumulates rapidly as the respiration intensity increases and then slowly decreases as the fruit matures and respiration subsides.

During the course of the first four or five days of ripening there are apparently acidic substances other than malic acid present in the fruit, as indicated by the excess of titratable acidity over the acidity due to malic acid. How much the pectic and tannic substances present in the unripe banana contribute toward this excess acidity is not known. However, it has been postulated, von Loesecke (1930), that pectic substances occur in a more or less insoluble condition in green fruit. During ripening the protopectin is enzymatically converted to pectin and finally into various semi-acid and acid bodies. The reverse

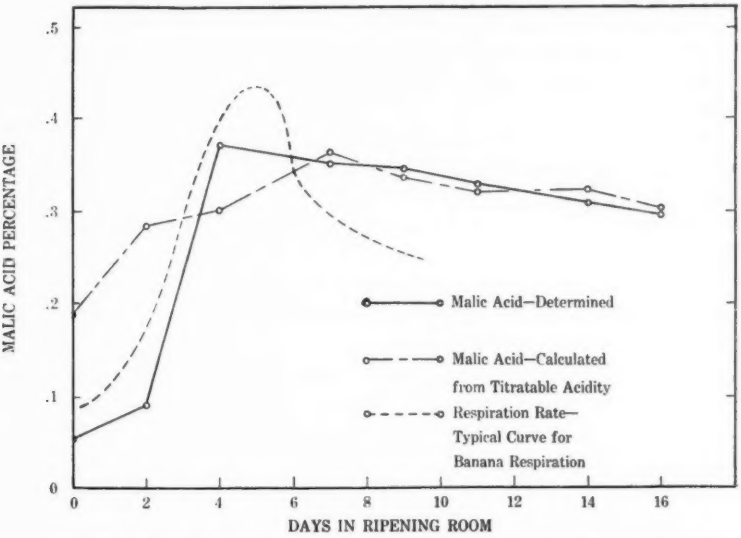

FIG. 1. Relationship between the malic acid content, the titratable acidity (calculated as percentage of malic acid), and the respiration rate of bananas during ripening.

may be the case with the tannins. The free tannins of the green fruit apparently become "bound" or "fixed" and have no demonstrable acidity as the fruit matures. The total amount, both of tannins and pectins, remains practically constant during ripening, the change being merely in their state, or form, of existence.

It seems evident that during the second half of the ripening period the titratable acidity is a measure of the malic acid content. Apparently in this period all of the malic acid in the banana is present as the free acid. This fact is interesting in the light of Sherman's (1934) statement that the mild organic acids occurring free in raw fruit are extremely effective in oral hygiene.

CONCLUSIONS

1. The principal nonvolatile organic acid of the ripe banana is l-malic acid. This apparently occurs as free acid in the ripe fruit. Other nonvolatile organic acids, if present at all, occur in extremely small amounts.

2. During ripening of the banana the l-malic acid content increases to a peak, then very gradually decreases as the fruit matures.

3. The range of variation of the l-malic acid content of the banana over the ripening period is from .053 to .373 per cent with a value of approximately .314 per cent for the stage of ripeness at which the fruit is usually eaten.

4. The titratable acidity of bananas varies during ripening from approximately 2.8 (ml. of N. alkali per 100 gm. of banana) to 5.4, with a value of approximately 4.8 for ripe fruit (yellow peel flecked with brown).

5. Titratable acidity is a measure of the l-malic acid content of bananas from the stage of ripeness at which the peel is more yellow than green to the dead-ripe stage.

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RELATION OF ACIDITY OF MILK TO OXIDIZED FLAVOR

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(Received for publication, March 29, 1937)

INTRODUCTION

It is generally conceded that "oxidized" or "cardboardy" flavor of milk is one of the most harassing problems which confronts the milk industry today. Hence many investigators have, with varying success, spent much time seeking the cause and remedy of this off-flavor. For this information the reader is referred to the list of references at the end of this paper, with the exception of Sommer (1935). To date, no practical solution of the problem has been presented which will always prevent the development of oxidized flavor in pasteurized milk.

Since 1926, during the winter months, the milk produced by cows in the Connecticut State College herd has often developed a pronounced oxidized flavor upon pasteurization. This flavor has been most pronounced during the period when the cows were fed dry roughages (from October to May).

The authors have failed to prevent the development of the oxidized flavor in pasteurized milk by following such recommended practices as:

1. Eliminating rusty pails and cans in handling milk.
2. Eliminating a vat with copper exposed and the substitution of a stainless steel vat.
3. Tinning all valves, pumps, and pipe lines.
4. Discarding the first 10, 20, or 30 gallons of milk which flows through the system.
5. Eliminating all dairy detergents in washing equipment.
6. Sterilizing all equipment with hot water at 82.2°C.(180°F.) instead of with chlorine.
7. Cooling freshly drawn milk rapidly or slowly.
8. Aerating freshly drawn milk.
9. Pasteurizing freshly drawn milk immediately.
10. Heating milk rapidly or slowly during the pasteurization process.
11. Cooling pasteurized milk rapidly or slowly in the vat or in the bottle.

12. Aerating pasteurized milk.

13. Increasing the number of lactic-acid-producing bacteria in the milk both before and after pasteurization.

14. Eliminating milk from certain cows.

Last fall this troublesome flavor defect recurred as usual. In an effort to eliminate the defect, *all* equipment which was not made of stainless steel or which was not properly tinned was given a new coating of tin. The oxidized flavor persisted.

In the course of recent experiments on oxidized flavor, milk from several small commercial milk plants was checked. It was found that one plant was never troubled with the development of oxidized flavor in pasteurized milk. It occurred to the writers that if the equipment was causing oxidized flavor in the college plant, then the milk from this commercial plant should develop the flavor if it were pasteurized and processed at the college plant. The milk so processed did not develop the oxidized flavor.

On the other hand, the milk from the college herd was pasteurized at the commercial plant and it developed an oxidized flavor within 24 hours. This seemed to eliminate the college processing equipment as the cause of oxidized flavor in the college milk.

The only variable noted between the milk from the college herd and the outside milk was a difference in acidity. The titratable acidity of the imported fresh raw milk was .14 per cent and that of the fresh raw milk from the college herd was .19 per cent. The difference in the titratable acidity of the milk indicated that the acidity might in some way be associated with development of the so-called oxidized flavor in the pasteurized milk from the college herd.

The experience of another commercial milk plant led the authors to a more serious consideration of acidity as the cause of the defect. This milk plant has kept a record of acidity of the milk from each of its 28 producers over a number of years. The average titratable acidity of the incoming milk from these producers is shown (Fig. 1). They, too, have been vexed by the development of oxidized flavor in pasteurized milk from late fall until late spring. Oxidized flavor developed in the milk on November 10 last fall and still persists. The titratable acidity of the milk is now relatively high. It is interesting to note that the oxidized flavor disappears in this milk on June 10, when acidity of the milk declines sharply. During the period from June 10 to November 10 the milk does not develop an oxidized flavor on pasteurization. During this period the acidity of the milk is relatively low. It should be borne in mind that the period from June 10 to November 10 does not mark the usual pasture limits in this area.

It is possible that there is a lag phase in the disappearance of the flavor in the spring and its advent in the fall in relation to the apparent acidity of milk.

It has been reported by Sommer (1935) that the titratable acidity of milk is higher in winter than in summer. This fact is substantiated by our own unpublished records.

If the observations that milk produced when cows are on pasture is low in acidity and that milk of low acidity produced in the winter months does not develop oxidized flavor on pasteurization are true,

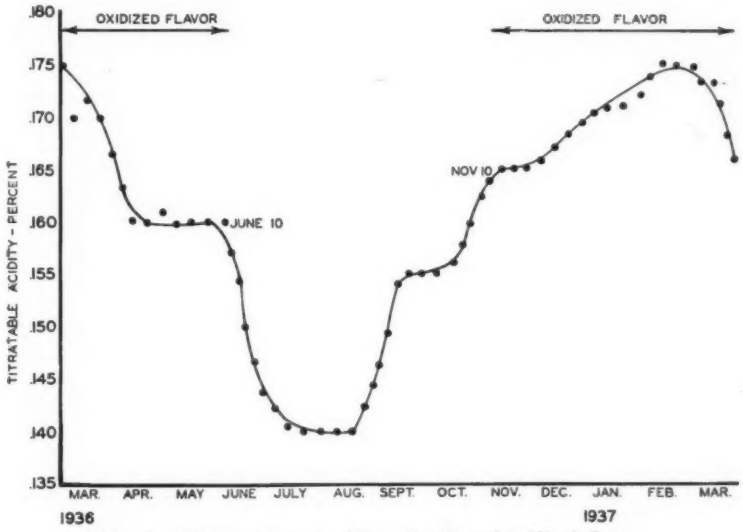

FIG. 1. Relation between acidity of milk and oxidized flavor.

then the neutralization of such high-acid milk should prevent the formation of this undesirable flavor. To this end the following experiments were conducted.

EXPERIMENTAL METHODS

The milk used in these trials was produced by the Connecticut State College herd and consisted of a mixture from animals of the Jersey, Guernsey, Holstein, and Ayrshire breeds. The milk was drawn by hand and strained into well-tinned 40-quart milk cans. The noon and night milkings were cooled to 4.4°C.(40°F.) in an electrically refrigerated milk tank. The morning milk was not cooled. All the milk was filtered through a cold milk filter into a 200-gallon stainless steel spray vat equipped with a diamond metal flush valve. All equipment was washed with lukewarm water and sterilized with water

heated to 82.2°C.(180°F.). Dairy detergents and chlorine were not used in cleaning or sterilizing the equipment.

The milk was pasteurized at 143°F. plus or minus .5°F. for 30 minutes, using water heated to 65.6°C.(150°F.) as a heating medium. The vat was equipped with an automatic control. After pasteurization the milk was cooled in the vat to 57.2°C.(135°F.) and then pumped through a newly tinned pipe line over a well-tinned cabinet cooler to the bottle-filler. Sweet water at a temperature of 7.2°C. (45°F.) was used as the sole cooling medium.

All samples were taken and stored in commercially washed and sterilized half-pint milk bottles. Raw-milk samples were taken from the vat after thorough agitation before the heating process was started. Samples from the vat were taken at the end of the heating and holding period and cooled in running water to 7.2°C. Samples of the first milk pumped over the cooler were taken, after which 10 to 20 gallons of milk were drawn off before opening the valve to feed the bottle-filler. The first six half pints from the filler were taken as samples. After 100 gallons of milk had been bottled, six more half-pint samples were taken. The last two groups of samples were commercially bottled and capped.

All samples were placed in the cooler at 4.4°C. (40°F.) and scored for flavor after 24, 48, and 72 hours. All flavor scoring was done on duplicate samples by three judges working independently. The data recorded is the average of the scores by the three judges. The following standard of scoring was used:

No oxidized flavor	0
Slight oxidized flavor	1
Oxidized flavor	2
Pronounced oxidized flavor	3
Very pronounced oxidized flavor	4

A score of 0 or 1 indicates saleable milk, 2 indicates a definite oxidized flavor, and a score of 3 or 4 indicates that the milk was not saleable.

The acidity was determined by titrating 17.5 c.c. of milk with N/10 NaOH using five drops of phenolphthalein as an indicator, and the results recorded were calculated as percentage of lactic acid.

A concentrated solution of Na_2CO_3 (CP) was used to neutralize the apparent acidity of the milk. The neutralizer was added prior to pasteurization at a temperature of from 26.7 to 32.2°C.(80 to 90°F.). The milk was held at this temperature for 10 to 15 minutes and the acidity again determined.

RESULTS ON HIGH-ACIDITY MILK

The results on the development of oxidized flavor in the unneutralized milk having a high apparent acidity are given (Table 1).

The raw milk did not develop an oxidized flavor within the 72-hour period. The pasteurized milk taken from the vat did not develop an oxidized flavor in 24 hours and only in one sample within 48 hours. Within 72 hours, however, milk in four of the seven trials developed an oxidized flavor.

TABLE 1

Development of Oxidized Flavor in Unneutralized Milk

Milk processed	1/24/37	1/27/37	1/31/37	2/3/37	2/12/37	2/28/37	3/7/37
Acidity of fresh milk (%)	.19	.19	.19	.19	.19	.19	.19
Source of sample	Intensity of flavor after 24 hours						
Raw	0	0	0	0	0	0	0
Past. from vat	0	0	0	0	0	0	0
Past. first over cooler	1	0	0	0	0	0	0
Past. first bottled	1	0	1	0	0	0	0
Past. last bottled	0	0	0	0	0	0	0
	Intensity of flavor after 48 hours						
Raw	0	0	0	0	0	0	0
Past. from vat	1	0	0	0	0	0	0
Past. first over cooler	2	2	2	2	1	0	1
Past. first bottled	1	2	3	1	2	1	1
Past. last bottled	0	1	0	2	1	0	0
	Intensity of flavor after 72 hours						
Raw	0	0	0	0	0	0	0
Past. from vat	1	1	1	2	0	0	0
Past. first over cooler	4	3	3	2	2	2	3
Past. first bottled	2	3	3	3	2	2	3
Past. last bottled	2	2	2	2	2	1	1

In one trial the first milk over the cooler did develop an oxidized flavor within 24 hours, and within 48 hours all except one trial developed the oxidized flavor. Within 72 hours all trials developed very pronounced oxidized flavor.

The first milk bottled developed a slight oxidized flavor within 24 hours in two out of seven trials. After 48 and 72 hours the first milk bottled was definitely oxidized.

The last milk bottled held up for 24 hours, but after 48 hours the trend was definitely towards an oxidized flavor. When the milk was 72 hours old, all of it was definitely off-flavor.

RESULTS ON LOW-ACIDITY MILK

At intervals the milk was neutralized below .15 per cent acidity. The effectiveness of this treatment in preventing the development of oxidized flavor is shown (Table 2).

None of the samples developed an oxidized flavor in 48 hours.

In only two out of seven trials did some of the samples of neutralized milk develop an oxidized flavor within 72 hours.

TABLE 2

Development of Oxidized Flavor in Neutralized Milk

Milk processed.....	1/22/37	1/28/37	2/5/37	2/9/37	2/11/37	2/27/37	3/5/37
Acidity of fresh milk (%).....	.185[1]	.185	.19	.19	.19	.19	.19
Neutralized to (%)...	.145	.145	.12	.125	.135	.12	.145
Source of sample	Intensity of flavor after 24 hours						
Raw.....	0	0	0	0	0	0	0
Past. from vat.....	0	0	0	0	0	0	0
Past. first over cooler	0	0	0	0	0	0	0
Past. first bottled.....	0	0	0	0	0	0	0
Past. last bottled.....	0	0	0	0	0	0	0
	Intensity of flavor after 48 hours						
Raw.....	0	0	0	0	0	0	0
Past. from vat.....	0	0	0	0	0	0	0
Past. first over cooler	0	0	0	0	0	0	0
Past. first bottled.....	0	0	0	0	0	0	0
Past. last bottled.....	0	0	0	0	0	0	0
	Intensity of flavor after 72 hours						
Raw.....	0	0	0	0	0	0	0
Past. from vat.....	0	0	0	0	0	0	0
Past. first over cooler	0	0	0	3	0	0	0
Past. first bottled.....	0	1	0	1	0	0	0
Past. last bottled.....	0	0	0	0	0	0	0

[1] This milk was neutralized with a mixture of salts and sugar, the estimated composition of which is:

Dextrose	91.23%	Sodium chloride	2.61%
Calcium oxide	2.99%	Water	.50%
Magnesium hydroxide	2.67%	Copper iron traces.	

Analysis made by Connecticut (New Haven) Agricultural Experiment Station.

RESULTS ON MEDIUM-ACIDITY MILK

An attempt was made to ascertain the degree of acidity above which pasteurized milk develops an oxidized flavor. This point seems to be at approximately .15 per cent titratable acidity for milk from the college herd as indicated by the results (Table 3).

Neutralizing the milk from the college herd to a titratable acidity of .15 per cent was not effective in prevention of the development of

oxidized flavor. The point below which oxidized flavor did not develop in the college milk was lower than that observed in the commercial milk plant. The two observations are not untenable because milks from different sources differ in their behavior. Hence it is to be expected that the acidity at which oxidized flavor entirely disappears in one milk plant may be higher or lower than that in another.

The authors do not advocate the neutralization of milk with sodium carbonate or any other neutralizing agent to control the development of oxidized flavor. The results are presented in the light that the effect of neutralization on retarding the development of oxidized flavor in milk may be explained on the basis of a balancing of the sodium-calcium-phosphorous-casein complex in milk. This in turn has led to a consideration of the mineral intake of the animals and its relationship to oxidized flavor. This phase of the problem is now being studied by the authors.

TABLE 3

Development of Oxidized Flavor in Neutralized Milk

Milk processed	1/29/37	1/30/37	2/10/37
Acidity of fresh milk (%)	.19	.19	.19
Neutralized to (%)	.15	.15	.15
Source of sample	Intensity of flavor after 24 hours		
Raw	0	0	0
Past. from vat	0	0	0
Past. first over cooler	0	0	0
Past. first bottled	0	1	0
Past. last bottled	0	0	0
	Intensity of flavor after 48 hours		
Raw	0	0	0
Past. from vat	1	0	0
Past. first over cooler	0	2	1
Past. first bottled	2	3	1
Past. last bottled	0	0	0
	Intensity of flavor after 72 hours		
Raw	0	0	0
Past. from vat	3	1	0
Past. first over cooler	1	2	1
Past. first bottled	3	2	2
Past. last bottled	2	1	0

CONCLUSIONS

1. Observations concerning oxidized flavor in pasteurized milk in a commercial plant indicated that the defect may be associated with the apparent acidity of milk.

2. Neutralizing high-acid milk to .145 per cent acidity or lower was effective in the prevention of oxidized flavor in pasteurized milk.

3. Neutralization to .15 per cent acidity did not prevent the development of oxidized flavor in milk in all cases.

4. Milk of high acidity invariably developed an oxidized flavor upon pasteurization.

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SPINACH AND KALE IN FROZEN PACK
I. SCALDING TESTS. II. MICROBIOLOGICAL STUDIES

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(Received for publication, April 12, 1937)

Although spinach is now being preserved and marketed successfully in frozen-pack form, very few experimental data have appeared on the microbiology involved. Furthermore, no published information is available on the suitability of freezing as a means of preserving kale for market. This paper describes the relationship between scalding and retention of quality in both spinach and kale, also the numbers and types of microörganisms present in the raw material and the effect of scalding and of freezing storage on this microflora.

EXPERIMENTAL MATERIALS

The spinach used in these experiments consisted of Bloomsdale Savoy and Viroflay varieties grown in Texas and Bloomsdale Savoy grown in nearby Virginia and Maryland. The Texas-grown spinach, which was in transit three days in refrigerated cars, was received at the laboratory in a chilled, crisp, high-grade condition and was packed and frozen about four hours after the cars were opened in Washington, D. C., on March 2, 8, and 22, 1934. Cooking tests on the fresh spinach showed that the flavor was very mild and sweet with little of the acridity or sharpness which often characterizes the flavor of fresh spinach.

Siberian kale, grown locally and picked the day before packing, was used in the preparation of the frozen packs of kale. The packing dates were May 9, 15, and 17, 1934.

It is well known that maturity plays a large part in retaining high grade in frozen-pack vegetables. This is especially true where kale is concerned, as only the young, tender leaves of this vegetable can be used for frozen pack. Spinach plants at three stages of maturity were used in these experiments: first, the young tender plants bearing leaves which were about full-sized but in which the flower stalks were rarely evident; second, plants on which the flower stalks were plainly visible but not elongated; and third, plants bear-

ing flower stalks which had begun to elongate but in which the flower buds had not begun to open.

I. SCALDING TESTS

Scalding, or blanching as it is sometimes improperly called, is an essential pretreatment for vegetables going into frozen pack if desirable color, flavor, and texture are to be retained. Scalding obviously offers many advantages in preparing vegetables for frozen pack, such as the saving of space owing to wilting and the reduction of microbial content; but one of the principal benefits derived is the partial destruction of those enzymes responsible for loss of flavor in raw vegetables. That enzymes are active even when foods are frozen has been pointed out by Diehl, Dingle, and Berry (1933), by Diehl and Berry (1933), and by other workers.

Techniques for scalding spinach have been the subject of many experiments. Arighi, Joslyn, and Marsh (1936) found that frozen packs of spinach which had been pretreated by scalding in water above 85°C.(185°F.) [90, 95, and 100°C.(194, 203, and 212°F.)] "were satisfactory in color and flavor and were equal to freshly cooked spinach in all respects." The scalding time was two minutes and their observations were made on packs held at —17.8°C.(0°F.) for over two years. According to Tressler and Evers (1936) commercial practice in scalding spinach preparatory to freezing generally follows the procedure suggested by Joslyn and Cruess (1929), namely, steam scalding at 100°C. for two to five minutes, followed by chilling in cold water or cold air, and packing in dilute brine.

Scalding in steam would appear to be superior to scalding in water as far as spinach and kale are concerned, because a large amount of valuable food material is lost from these plants as soon as they are plunged into hot water. Magoon and Culpepper (1924) have found that spinach scalded two minutes in boiling water loses from 16 to 30 per cent of its total dry matter. They also state that chilling spinach in cold water is an expensive and wasteful practice because it allows a still further loss of food value.

The scalding period for leafy vegetables should be long enough to inactivate enzymes responsible for deterioration and yet not long enough to soften the texture to such an extent that further cooking will make the vegetables too soft to be palatable.

Destruction of catalase in the tissues of peas has been found by Diehl, Dingle, and Berry (1933) to be an index to the destruction of other enzymes possibly more concerned than catalase in the spoilage of peas. These workers suggested the tests for the presence of catalase used in these experiments, namely, the evolution of oxygen from

hydrogen peroxide in the presence of plant tissues. It was found by these workers that the scalding period must be extended until the test for catalase in the tissues is either very weak or negative before high quality in the frozen-pack peas can be expected.

Results of the preliminary tests of scalding for the inactivation of enzymes as indicated by catalase destruction in spinach and kale show that catalase and presumably other enzymes were nearly but not completely inactivated by scalding spinach for three or five minutes and kale for five minutes (Table 1). Since complete destruction was considered unnecessary, and since a seven-minute treatment produced too much softening of the tissues, three- and five-minute scald-

TABLE 1

Tests for Catalase Activity in Steam-Scalded Spinach and Kale as Shown by the Hydrogen Peroxide Method[1]

Temperature	Time	Spinach	Kale
	min.		
100°C.(212°F.)	1	+	++
100°C.(212°F.)	3	±	+
100°C.(212°F.)	5	±	±
100°C.(212°F.)	7	—	—

[1] Suggested by Diehl, Dingle, and Berry (1933).
++ = strong catalase activity.
+ = moderate catalase activity.
± = weak catalase activity.
— = no catalase activity.

ing periods were used in the preparation of the experimental packs of spinach and kale.

PACKING IN TIN CANS AND PAPER CUPS

After a careful washing and trimming the spinach or kale was weighed out into one-pound lots. Each lot was spread about two inches deep on a shallow tray, placed in the steamer, and scalded at 100°C. (212°F.) for three or five minutes. The steam entered horizontally over the greens about one inch above them. After scalding, part of the material was chopped into fairly small pieces in a manner similar to the way spinach or kale is cut before serving. Both chopped and unchopped leaves were packed in three different ways: without added liquid; after mixture with 100 c.c. of two-per cent NaCl solution; after mixture with 100 c.c. of a .24-per cent solution of $CaCl_2$ (used only in the spinach packs). The addition of the $CaCl_2$ solution resulted in the immediate formation of a fine precipitate of calcium oxalate crystals that was evident in the liquor; inclusion of part of this precipitate in the pack showed no undesirable effects.

The purpose of adding a dilute solution of $CaCl_2$ to a part of the spinach pack was to determine whether the "furring" or roughening

of the teeth by cooked spinach noted by many consumers could be prevented. If this is due to the action of free oxalic acid on the teeth, the effect might be avoided by precipitating the oxalic acid as calcium oxalate; furthermore, any undesirable flavor owing to the action of free oxalic acid might be eliminated at the same time. In addition it may well be reasoned that the colloidal properties of the cell wall may be altered to give improved texture by altering the ratio of divalent ions, such as Ca, and monovalent ions, such as Na and K. Some improvement does result which will be discussed later.

After the various treatments the greens were packed in either No. 1 tin cans (Picnic or Eastern) or paraffined paper pint cups. One pound of fresh spinach, after being steam-scalded, filled a No. 1 tin can, allowing one-half inch head space for expansion owing to freezing. When 100 c.c. of brine or $CaCl_2$ solution were added, there was some excess liquid which was discarded. Slightly more than one pound of fresh spinach was needed to properly fill the paper cups. Where the paper containers were used, the greens were first cooled in a refrigerator. The tin cans were hermetically sealed and the paper cups covered, but the paper covers were not air-tight. The packs in tin cans were placed at once in contact with dry ice in small portable refrigerators at approximately —73.4°C.(—100°F.), while those in paper cups were placed in electric refrigerators at approximately 4.4°C.(40°F.). Within four hours all the packs were transferred to cold storage at —9.4°C.(15°F.). The prompt and thorough cooling of all the packs of spinach and kale is held responsible for the fact that this material held up so well in flavor, color, and texture at a storage period of —9.4°C. It is to be noted that Diehl, Pentzer, Berry, and Asbury (1934) recommended —17.8°C.(0°F.) as the optimum freezing and storage temperature for most vegetables. Recent work by Moon, Caldwell, and Lutz (1936); Caldwell, Lutz, Culpepper, and Moon (1936); Moon, Caldwell, Lutz, and Culpepper (1936); and Caldwell, Lutz, and Moon (1936) has shown that peas, corn, snap beans, and lima beans, packed under conditions more nearly resembling commercial packing than was the case in the work reported here, require a storage temperature of —17.8°C. to insure high quality in frozen packs.

QUALITY OF PACKS OF SPINACH

After storage periods of varying lengths, samples of frozen spinach were withdrawn for cooking tests. The packs were put directly into boiling salted water without thawing and actually boiled for eight to ten minutes. Judging was done by several workers engaged in food utilization studies in the U. S. Department of Agriculture laboratories.

Opinions of the judges on the quality of the experimental packs is shown (Table 2).

In general, spinach steamed three minutes was judged to be better in flavor, color, and texture than that steamed five minutes which was more or less insipid in flavor and less attractive in color and texture. The immature spinach, steamed three minutes and packed without added liquid, received the highest rating of any of the packs after a storage period of seven months at —9.4°C.(15°F.).

Spinach packed in two-per cent brine did not grade as high as that without brine or that treated with .24-per cent $CaCl_2$ solution. The spinach treated with $CaCl_2$ was equal in quality to the untreated packs where the younger plants were used and stored for shorter periods, and was slightly better in flavor and texture where the overmature plants were used, especially after holding for 15 months in storage. It had a milder flavor than the untreated and caused less "furring" or roughening of the teeth. Some improvement in texture was noted in all the spinach treated with $CaCl_2$ in that it was firmer after the cooking tests.

While results as indicated were consistent in these experiments, the use of $CaCl_2$ as a packing medium is not recommended at present in commercial packs; further work in other laboratories must be conducted to test the practicability of this suggestion. The effect of pretreating with $CaCl_2$ on the vitamin-C content is not known but should be investigated. The toughening effect of "hard water" containing calcium and magnesium salts on peas and lima beans is, of course, well known to packers. We wish to emphasize that the same effect does not necessarily extend to the processing of all leafy vegetables.

Chopping spinach before packing helped to retain slightly more of the fresh green color and made the cooking process somewhat easier.

Very little difference in quality was observed in spinach packed in tin cans or in paper cups. There is a tendency for the top layer of spinach frozen and stored at —9.4°C. in paper cups to become withered and unattractive in appearance. The tighter the seal, the less likely this is to happen.

It is not possible to explain at present why the spinach grown locally was not successful in frozen pack. Variety, length of growing season, weather conditions during growing season and at harvest time, and other factors are known to affect the suitability of vegetables for frozen pack. It may be that some of these factors were responsible for this lack of success or it may be that a different scalding schedule or a lower storage temperature would result in better quality packs.

TABLE 2

Quality of Cooked, Frozen-Pack Spinach after Storage Periods of Approximately Six Months and One Year at —9.4° (15° F.)

Types	Storage period	Rated according to	3 minutes steam-scalding			5 minutes steam-scalding		
			Without brine	2% NaCl added	.24% $CaCl_2$ added	Without brine	2% NaCl added	.24% $CaCl_2$ added
	mo.							
Texas grown		Color	Excellent	Excellent	Excellent	Fair	Good	Good
Bloomsdale Savoy and Viroflay	7	Flavor	Excellent	Fair	Good	Poor	Poor	Poor
Immature plants		Texture	Very good	Good	Excellent	Poor	Poor	Fair
Leaves full-sized		Color	Excellent	Excellent	Excellent	Fair	Poor	Fair
Very few flower stalks.	15	Flavor	Excellent	Good	Excellent	Good	Good	Good
		Texture	Good	Fair	Very good	Good	Good	Very good
		Color	Excellent	Good	Excellent	Good	Good	Poor
Texas grown	9	Flavor	Good	Good	Good	Fair	Fair	Fair
Fully mature plants		Texture	Very good	Fair	Excellent	Good	Fair	Very good
Flower stalk well defined on		Color	Good	Good	Good	Fair	Fair	Good
nearly all plants.	13	Flavor	Good	Good	Excellent	Fair	Good	Good
		Texture	Good	Fair	Excellent	Fair	Fair	Good
Texas grown		Color	Excellent		Excellent	Good		Excellent
Overmature plants	9	Flavor	Good	Not	Good	Good	Not	Good
Flower stalks on most plants elongated.		Texture	Fair	packed	Good	Good	packed	Very good
		Color	Good		Good			
(These were removed before	15	Flavor	Good	Not	Excellent	Not	Not	Not
packing.)		Texture	Good	packed	Excellent	packed	packed	packed
		Color	Poor	Poor	Poor		Poor	
Virginia and Maryland grown	7	Flavor	Fair	Poor	Fair	Not	Fair	Not
Bloomsdale Savoy		Texture	Good	Fair	Very good	packed	Poor	packed
Majority of plants immature—		Color						
not well graded for maturity.	12	Flavor	Not	Not	Not	Not	Not	Not
		Texture	edible	edible	edible	edible	edible	edible
		Color	Fair	Good	Good			
Virginia and Maryland grown	8	Flavor	Good	Good	Good	Not	Not	Not
Bloomsdale Savoy		Texture	Poor	Fair	Good	packed	packed	packed
Various stages of maturity.		Color						
	12	Flavor	Not	Not	Not	Not	Not	Not
		Texture	edible	edible	edible	packed	packed	packed

QUALITY OF PACKS OF KALE

The flavor, texture, and color of kale appears to be less affected by steaming than is the case with spinach; in fact, the flavor of five-minute steam-scalded kale was superior in all respects to that scalded only three minutes. It may be that even longer scalding periods would be desirable. Kale packed without brine was superior to that packed in brine, the brine pack having a tendency to become brown. This tendency was slight and was not noticeable after the product was cooked.

Kale is less changed in color, flavor, and texture after being preserved by freezing than many other vegetables. Some of the judges expressed the opinion that there is improvement in the flavor of cooked frozen kale over cooked fresh kale.

It is interesting to note that under the conditions in this experiment a direct correlation seemed to exist between the number of minutes steaming required to inactivate catalase in the tissues of spinach and kale (indicated by $\pm$ in Table 1), and the number of minutes steaming or scalding necessary before freezing to insure the best quality in the frozen packs, namely, three minutes for spinach and five minutes for kale. Longer or shorter scalding periods may be required for optimum retention of quality in commercial practice depending upon the volume of material introduced into the scalder per unit of time.

II. MICROBIOLOGICAL STUDIES

James (1932), Wallace and Tanner (1933), and Berry and Magoon (1934) have reviewed the literature dealing with the effect of low temperatures on growth and survival of microörganisms. The subject is treated by these authors not only from the food-storage angle but also from the viewpoint of the effect of low temperatures on the microbial content of water, soil, and various other substrata both organic and inorganic.

Berry (1933) reports the presence of organisms of the "colon" group, chiefly of the genus *Aerobacter*, in spinach scalded at 95°C. (203°F.) for one to three minutes and stored at —10°C.(14°F.) for ten months.

Brown (1933) found that defrosted frozen-pack spinach spoiled more rapidly at 6 and 22°C.(42.8 and 71.6°F.) than either defrosted peas or lima beans, but that all three spoiled after 36 to 48 hours at 6°C. and after 12 to 24 hours at 22°C. She reports the isolation of four genera of bacteria from fresh vegetables and as many from frozen vegetables.

Prescott (1932) gives microbial counts on spinach quick-frozen at —29°C.(—20.2°F.) and held at —6.6, —12, and —18°C.(20.1, 10.4 and —0.4°F.) for 1, 2, 4, 6, 8, 10, and 12 weeks. He reports an initial

TABLE 3

Microbial Content per Gram of Spinach and Kale

Fresh spinach trimmed and washed	Steam-scalded	Frozen and stored at —9.4°C.(15°F.) approximately 7 months			Frozen and stored at —9.4°C.(15°F.) approximately 15 months		
		Without brine	In brine	In $CaCl_2$ sol.	Without brine	In brine	In $CaCl_2$ sol.
Texas grown 2,000,000	3 minutes 2,000	400	400	350	75	200	150
	5 minutes 150	100	50	50	100	100	150
Texas grown 23,000,000	3 minutes 12,000	600	500	200	200	100	150
	5 minutes 1,800	120	?	100	Less than 5	20	100
Locally grown 5,000,000	3 minutes 1,600	200	150	150	200	1,000	400
	5 minutes 240	20	4,500	150	?	?	100
Fresh kale trimmed and washed Locally grown 1,200,000	3 minutes 3,000	500	500	Not packed	200	300	Not packed
	5 minutes 1,500	200	150	Not packed	50	150	Not packed
Locally grown 10,000,000	3 minutes 7,500	400	350	Not packed	100	200	Not packed
	5 minutes 2,000	50	10	Not packed	50	50	Not packed

count of 2,170 per gram of spinach after quick-freezing at —29°C. (—20.2°F.) and states that this count remains about the same when the packs are held at —18°C. for 12 weeks; while, although fluctuations occur, the count is reduced to half that number in packs held at —12°C. and —6.6°C. He concludes that in certain foods the decreases in numbers of bacteria occur more rapidly and to a greater degree at the higher storage temperatures.

Lochhead and Jones (1936) reported that freshly packed vegetables (asparagus, peas, beans, and corn) have a microbial content subject to wide variation, but these variations tend to become obliterated during storage at —17.8°C.(0°F.). They also report that in the vegetables studied species of *Micrococcus* and *Flavobacterium* withstand nine months' freezing better than other types of bacteria.

Smart (1934) found that the microbial content of frozen-pack spinach packed in many localities, some of it having an unknown storage history, varied from 57,000 to over a million per gram. These high counts are at variance with findings in the present study and may have been caused by insufficient heat penetration during pretreatment by scalding or by too-high storage temperature. She names five species of bacteria repeatedly isolated from frozen spinach. No counts are given on the spinach before freezing.

Wide variation in microbial content of fresh and frozen vegetables was reported in a study of lima beans in frozen pack by Smart and Brunstetter (1936).

EXPERIMENTAL METHODS AND DATA

Spinach and kale were sampled for bacteriological analysis after they were trimmed and washed, again after they were scalded, and finally at each time the cut-out tests were made on the frozen material. Samples consisted of 100 grams selected from many parts of the mass in order to secure material that would be representative. These samples were mixed with 100 c.c. of sterile tap water and thoroughly agitated. After proper dilutions were made, plates were poured, using beef agar adjusted to pH 7.0 for bacteria and wort agar at approximately pH 4.5 for molds and yeasts. Beef-agar plates were incubated at room temperature, 27 to 30°C. (80.6 to 86°F.), for two days while the wort-agar plates were incubated somewhat longer to allow the molds to sporulate.

After the numbers of microörganisms per gram were determined, the predominating types were transferred to agar slants and run through the tests for species identification.

The microbial count per gram of material at each stage in the preparation and cutting of frozen-pack spinach and kale is given (Table 3). Under the conditions of this experiment the microbial

TABLE 4

Microörganisms Repeatedly Isolated from Spinach and Kale

Fresh and scalded spinach and kale	Frozen-pack spinach and kale after several months' storage at —9.4°C.(15°F.)
Bacteria:	Bacteria:
Achromobacter salopium (Grey & Thornton) Bergey, et al.	*Bacillus albolactis* Migula
Achromobacter radiobacter (Beijerinck & van Delden) comb. nov.	*Bacillus cereus* Frankland and Frankland
Aerobacter aerogenes (Kruse) Beijerinck	*Bacillus mesentericus var. flavus* Laubach
Bacillus albolactis Migula	*Bacillus mycoides* Flügge
Bacillus cereus Frankland and Frankland	*Bacillus niger* Migula
Bacillus mesentericus var. flavus Laubach	*Bacillus ruminatus* Gottheil
Bacillus mycoides Flügge	*Bacillus subtilis* Cohn emend. Prazmouski
Bacillus niger Migula	*Bacillus vulgatus* Trevisan
Bacillus prausnitzii Trevisan	*Flavobacterium acetylicum* Levine and Soppeland
Bacillus subtilis Cohn emend. Prazmouski	*Flavobacterium breve* Frankland and Frankland
Bacillus vulgatus Trevisan	*Flavobacterium harrisonii* Bergey, et al.
Chromobacterium sp.	*Flavobacterium solare* (Lehmann u. Newmann) Bergey, et al.
Flavobacterium acetylicum Levine and Soppeland	*Micrococcus candidus* Cohen
Flavobacterium harrisonii Bergey, et al.	*Micrococcus cremoides* Zimmerman
Flavobacterium sulfureum (Lehmann u. Newmann) Bergey, et al.	*Pseudomonas* sp.
Micrococcus cremoides Zimmerman	*Pseudomonas fluorescens* Migula
Phytomonas sp.	*Pseudomonas putida* Migula
Pseudomonas fluorescens Migula	*Rhodococcus agilis* (Ali-Cohen) Holland
Sarcina sp.	*Sarcina* sp.
Fungi:	*Sarcina citrea* (Migula) Bergey, et al.
Aspergillus sp.	*Serratia* sp.
Cladosporium sp.	*Staphylococcus* sp.
Fusarium sp.	Fungi:
Monilia sp.	*Aspergillus* sp.
Mucor sp.	*Cladosporium* sp.
Penicillium sp.	*Monilia* sp.
Peronospora sp.	*Mucor* sp.
Rhizopus sp.	*Penicillium sp.*
Yeasts:	*Rhizopus* sp.
Mycoderma sp.	Yeasts:
Saccharomyces, several sp.	*Mycoderma* sp.
Torula sp. (black, possibly nigra)	*Saccharomyces,* several sp.
Torula sp. (red)	*Torula sp.* (black, possibly nigra)
	Torula sp. (red)

content of fresh spinach and kale is reduced over 99 per cent by steam-scalding for three and five minutes and is in most cases further reduced by freezing and storage. Although there is wide variation in the microbial count of the different lots of fresh material, probably due to weather conditions at harvesting time, these differences are not directly reflected in the counts on the frozen material, particularly after storage for 15 months. The variation in counts on individual cans is wide, but in general the microbial content of the frozen packs is low. The counts on a few of the brine packs of spinach are much higher than the average but otherwise no differences were evident in their quality. Where proper care is exercised in trimming and washing spinach and kale and when prompt cooling and freezing are insured, high counts in the frozen packs of these products should not be encountered.

MICROBIAL FLORA OF FRESH AND FROZEN SPINACH AND KALE

Considering the manner in which spinach and kale grow and their close proximity to the soil, it was expected that their microbial content would be largely made up of the common soil types. This proved to be true, but there were other sources of contamination before the material was ready to be packed, such as wash water, various containers, and hands of pickers and packers. Some of these sources of contamination may explain the many species of bacteria isolated from the frozen spinach and kale that were not found in the fresh materials. The predominating types of bacteria in fresh spinach were distributed among four genera: *Achromobacter*, *Bacillus*, *Flavobacterium*, and *Pseudomonas*. The same genera of bacteria are found in abundance on fresh kale in about the same proportions. Yeast and mold counts on kale used in these experiments were higher than those on the spinach, but the same types were found on both products. The sugar content of kale is 1.19 per cent while spinach has .6 per cent, according to Chatfield and Adams (1931). The higher yeast and mold counts in kale may be explained by the higher sugar content in that product. Lists of microörganisms found on fresh and frozen spinach and kale are given (Table 4).

In giving the names of species of the various genera of bacteria it is necessary to explain that some of the cultures isolated in this study vary in a few of their characteristics from the species as described in the literature. While some variations may warrant new names of species for these organisms, most of them do not.

A wide diversity of microbial species were isolated from frozen spinach and kale, but these types are those ordinarily found on fresh vegetables and in no way affect the wholesomeness of the product,

provided that in cooking the vegetables they are placed in boiling water prior to or immediately after defrosting.

SUMMARY

Frozen-pack spinach and kale were prepared for the purpose of determining the scalding technique necessary to insure maximum retention of quality in these vegetables, and also to determine the behavior of microörganisms normally present in spinach and kale at different stages in the preparation and storage of the frozen packs.

Of one-, three-, five-, and seven-minute pretreatments under the conditions of this experiment, three-minute scalding in steam was found to be best adapted to the retention of high quality in frozen-pack spinach, while five-minute steam-scalding best retained quality in frozen-pack kale. The amount of material introduced into the scalder per unit of time will necessarily affect the length of the scalding period.

A dilute solution (.24 per cent) of $CaCl_2$ added to blanched spinach before freezing resulted in better texture, color, and flavor in some of the packs; but no definite conclusions can be drawn concerning its probable value as a commercial practice at this time.

Two-per cent brine did not prove a satisfactory packing medium.

While a storage temperature of —9.4°C.(15°F.) was found to be sufficiently low to protect the majority of these experimental packs from loss of desirable color, flavor, and texture, —17.8°C. (0°F.) is a safer temperature for commercial packing, particularly for long storage periods.

Wide variation in microbial content was noted in different lots of both fresh and frozen spinach and kale.

Scalding, freezing, and storage at —9.4°C. reduced the microbial content of the spinach and kale in this experiment more than 99 per cent.

While the microbial content of the frozen greens was characterized by a great diversity of types, the counts were very low and the types present were those ordinarily found on fresh vegetables. In no way do they affect the wholesomeness of the product, provided that in cooking the vegetables are placed in boiling water prior to or immediately after defrosting.

Where proper care is taken in handling good-quality spinach and kale and where prompt cooling and freezing are practiced, high microbial counts should not be encountered in the frozen packs of these products.

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THE STALING OF COFFEE, II [1]

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(Received for publication, April 25, 1937)

An earlier paper on this subject (Food Research Vol. 2, No. 1) described work on the isolation and identification of chemical constituents obtained from roasted coffee and believed to be responsible factors in the flavor and aroma of the freshly roasted bean and of the beverage produced therefrom. This work gave such promise of interesting and worth-while results that a continuation along the same general lines of investigation was conducted.

REVIEW OF EARLIER WORK

Previous experiments were made on concentrated aqueous coffee decoctions made from quantities of roasted coffee ranging from 25 to 50 pounds. Continuous gravity extraction of these, first with ether, then with ethyl acetate, gave deeply colored solutions which in the earlier work were concentrated by fractionation of the solvents at barometric pressure.

Concentration of the ether extracts usually gave a deep-brown, evil-smelling, somewhat viscous oil which, in each case, was submitted to a high-vacuum distillation (2 to 3 mm.) in order at once to separate volatile and non-volatile materials, without attempt at fractionation. The distillate was usually a limpid liquid, colorless or slightly yellow, which on standing, especially in open vessels, rapidly assumed a deep-yellow to brown color. Fractionation *in vacuo* of these distillates led to the identification of the following substances:

Furfuryl alcohol, identified by producing from it alpha-napthyl isocyanate, is a colorless liquid of fragrant odor and which exhibits a marked tendency towards polymerization and resinification on standing, especially in aqueous solution.

Furfuraldehyde, identified as its dimethyl dihydroresorcinol condensation product, is the oxidation product of furfuryl alcohol. It is an odoriferous liquid which promptly undergoes decomposition with the formation of tarry materials.

Acetic and formic acids were identified through their p-bromphenacylesters. The higher, more deeply colored fractions were shown

[1] Contribution No. 94 of the Department of Biology and Public Health. This work was made possible by a grant from the American Can Company.

to contain diacetyl by precipitation of the characteristic nickel derivative of diacetyl dioxime.

Similar results were obtained by the fractionation of the concentrates from the ethyl acetate extractions. Furfuryl alcohol, furfuraldehyde, acetic and formic acids were easily identified.

The very tarry nature of the acetate concentrates made it impossible, in one case, to fractionate the residue without further treatment. The tarry mass was therefore digested with strong sodium carbonate, reprecipitated with hydrochloric acid, and the tarry product taken up in ether. Fractionation of the concentrate from this ether solution yielded, in addition to the furane compounds obtained in the other experiments, diethyl ketone. This substance, identified by producing its 2, 4 dinitrophenyl hydrazone, is a colorless liquid not unlike acetone in its chemical and physical properties.

EXPERIMENTAL PROCEDURE

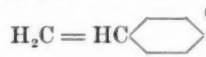
As the possibilities of the procedure so far outlined were seemingly exhausted, the scope of the investigation was considerably enlarged. Since the material under examination would be expected to be sensitive to the action of heat, the long, slow concentration at a relatively elevated temperature might well be a deleterious process. In view of this danger it was decided to carry out the concentration at reduced pressure and consequently at room temperature or lower. To this end a rather elaborate, but very efficient, concentration apparatus was constructed. The device was a continuous vacuum still, embodying the principle of the Bogert fractional distillation receiver. A constant vacuum was maintained by a bimanometric bleed vacuum regulator developed during the course of the investigation. The chemical examination of the concentrates obtained by this new procedure was delayed until a quantity several times larger than that used in any of the previous experiments had been obtained. In one instance the concentrate from about 65 pounds of roasted coffee was examined.

The investigation of the ether extracts obtained by the new method yielded in addition to the compounds obtained by the older method a number of interesting results.

A crystalline, very stable, saturated, long-chain aliphatic hydrocarbon, n-heptacosane, was easily separated and identified by the physical properties—melting point and molecular weight determination—as well as by the chemical inertness of a highly purified sample. Although the great stability of this substance would seem to vitiate any speculation as to its physiological action, it is perhaps significant that very recent investigations tend to show that the hydrocarbon is a

concomitant of the sterols, wherever the latter are found. This is of interest as at least one sterol has been isolated from the non-saponifiable portions of coffee fat. This fact, coupled with the recently accomplished biosynthesis of sterols from long-chain aliphatic acids, may lend to this fundamentally inactive substance a considerable but as yet unpredictable interest.

The higher boiling fractions of this ether concentrate were subjected to a vigorous steam distillation. Extraction of the distillate with ether, followed by concentration of the ether solution, gave an oil possessing an odor reminiscent of guaiacol. Distillation of this oil twice in the molecular still followed by fractionation in ordinary vacuum gave a colorless oil of guaiacol odor and sharp taste resembling that of eugenol. The substance is unsaturated and turns brown on exposure to air. It gives pronounced ferric chloride tests of such character as to indicate, with the other facts, the structure



From reasoning which will be apparent from the later work, it is probable that this substance is p-vinyl guaiacol.

The aqueous solution from steam distillation, after the extraction with ether, was then examined. This solution exhibited, in addition to pronounced ferric chloride colorations, strongly reducing properties indicating an aldehyde, a sugar, or a polyhydric phenol. Since the solution gave no aldehyde test, and the specific test for furfural fails, and since the material has already been submitted to a vacuum distillation, the first two possibilities are excluded. The assumption of the presence of a polyhydric phenol is rendered very probable by the relatively great insolubility in water. The facts tend to show that the material is p-vinyl catechol, but the extremely sensitive nature of the compound coupled with the absence of suitable derivatives and the minute amounts present have made impossible its positive identification.

The very high boiling fractions of the ether concentrate crystallized in the sidearm of the distilling flask and were shown to consist only of caffeine.

Attention was then turned to the investigation of the corresponding ethyl acetate concentrates. In addition to furfuryl alcohol, furfural, acids, caffeine, and diethyl ketone, a high-boiling phenolic fraction was obtained. The ferric chloride colorations obtained from this "acetate phenol" were quite different from those obtained from the ether concentrates, and gave tests similar to those described by Bernheimer for a phenolic oil isolated from roasted coffee beans by steam

distillation. Unfortunately, in no case could enough of the material be obtained to effect its separation in a state of purity. In the course of the work, however, the phenol was found to possess considerable volatility with steam even *in vacuo*.

Attempts at benzoylation of the crude phenolic mixtures by the Schotten-Baumann method yielded a benzoate, M.P. 106°C.(223°F.), crystallizing in beautiful, fine, silky needles. This substance is probably the benzoyl derivative of 4-hydroxy 3-methoxy acetophenone, or vanillone, a substance which sublimes readily and whose properties agree closely with those of solutions obtained by the steam distillation of the crude phenolic mixture.

Since all the residues obtained in the above experiments on the identification of phenolic constituents were contaminated with considerable amounts of caffeine, which might well hinder the detection of other crystalline substances, extractions were run on decaffeinated coffee.

The results obtained agreed very well in substance with those already obtained from extraction of ordinary coffee. Certain differences in quantity could be detected when correlative experiments were run, e.g., a considerable portion of the fats seemed to have been removed from the decaffeinated products. The prediction that the chemical examination of the higher boiling fractions would be simplified was found to have been justified. All efforts, however, to obtain a pure sample of the phenol described by Bernheimer were still unsuccessful even though qualitative tests indicated its presence very strongly.

EXAMINATION OF FRESHLY ROASTED GROUND COFFEE

In an effort to increase the possibilities of the investigation, work was begun on the direct ether extraction of the freshly roasted and ground coffee bean. It was found most advantageous to use decaffeinated coffee since the ratio of fats to other materials was found to be much smaller in it than in ordinary coffee.

In this work the concentrates, obtained by the usual method, were directly precipitated with methyl alcohol in order to free the desired constituents from a large portion of the fats with which they were admixed. The vacuum concentrations of the methanolic extracts obtained in this way led to deep-colored, viscous liquids of fine coffee odor which in the first experiments were distilled directly *in vacuo*, sometimes with great difficulty. The further treatment of the distillate led to the isolation, as main product, of a highly refractive oil of pleasant odor resembling that of certain of the terpenes. Since the latter possess approximately the same volatility as furfuryl alcohol, the separation of the two is a matter of considerable difficulty. It may be

this coincidence to which the failure of other investigators to obtain terpene-like compounds may be ascribed.

The fortuitous application of the Wallach test for sylvestrene gave a positive result, thus substantiating the belief that the above-mentioned liquid was a mixture of terpenes. As the latter are volatile substances possessing taste and odor and sensitive in the highest degree to heat, light, air, and chemical reagents, their identification would be a matter of great importance where the flavor and aroma of coffee are in question.

In an attempt at a more facile method of isolation of the terpene mixture, vacuum distillation was abandoned for a vigorous steam distillation of the concentrate from the ether extraction. Extraction of the distillate with ether and concentration of the solution so obtained led to the isolation of relatively large amounts of n-heptacosane, in addition to a beautifully crystalline substance, M.P. 117 to 118°C. (242.6 to 244.4°F.), whose positive identification has not yet been accomplished, and the desired volatile substances. It is worthy of note that in great dilution the concentrates obtained by this procedure, quite unlike those formerly obtained, possessed a fine coffee aroma.

In view of the hydrocarbon nature of the terpenes and possibly also of the crystalline material, M.P. 117 to 118°C.(242.6 to 244.4°F.), is was decided to substitute benzene for ether as extractive. This has the added advantage that a relatively small proportion of fats is obtained.

Further examination of the crystalline compound to which reference has been made in the foregoing paragraphs showed the insolubility of this material in cold concentrated sulphuric acid. This fact, coupled with tests made under the microscope which gave evidence of its forming a picrate, led to the conclusion that it was probably an aromatic hydrocarbon. It seemed likely that more of it might be obtained by a benzene extraction. Accordingly, a few grams of roasted, decaffeinated coffee was extracted with benzene in a Soxhlet extractor; n-heptacosane was obtained in the usual manner. Evaporation of the benzene solution left two kinds of crystals, the first of which turned violet on standing. This suggested the "kahweol" reported by Bengis and Anderson. Recrystallization from methyl alcohol gave very few crystals of a white substance, M.P. 140°C.(284°F.), tending to confirm the suspicion that the substance obtained was indeed kahweol, M.P. 142°C.(287.6°F.). This material has not previously been obtained in such manner. Together with the kahweol a minute quantity of material melting at 111°C.(231.8°F.) under the microscope was obtained. Apparently it was the desired hydrocarbon. This material was the same as that mentioned earlier

as having a melting point of 117 to 118°C. The latter determination was made in the usual manner, while results made by the method involving the use of the microscope are consistently lower than those by the other method, generally from 5 to 6 or even 7°C.

In view of the success of this first benzene extraction, a large-scale extraction was carried out. Although great difficulty was experienced, concentration of the extract was finally effected by evaporation under reduced pressure in a stream of carbon dioxide—a long and tedious process. The concentrate separated into two layers, each of which was steam distilled separately.

The steam distillate from the upper layer was extracted with ether, the ether concentrated, and the concentrate subjected to distillation at 10 mm. pressure. Three separate liquid fractions were obtained, together with a solid which proved to be n-heptacosane, M.P. 54 to 56°C.(129.2 to 132.8°F.). The high-boiling fractions were subjected to low-pressure distillation in the molecular still. Three fractions were isolated, and each fraction extracted with alkali and benzoylated separately by the Schotten-Baumann procedure. The following benzoates were isolated:

(1) 4 Hydroxy-3 methoxy styrene benzoate (p-vinyl guaiacol benzoate), from the high-boiling fraction, M.P. 111°C.(231.8°F.)

(2) Eugenol benzoate, M.P. 69°C.(156.2°F.)

(3) Guaiacol benzoate, M.P. 57°C.(134.6°F.).

The identity of the latter two benzoates was confirmed by mixed melting-points with samples synthesized from the phenols and benzoyl chloride. In addition color tests were obtained indicating the presence of p-vinyl catechol. No derivative of this substance is known.

The fractions insoluble in alkali were combined and redistilled *in vacuo*. The fraction which corresponded in boiling-point to furfuryl alcohol was washed with water immediately after distillation to remove that alcohol, and the chloroform solution of the water-insoluble portion was brominated at once with a dilute solution of bromine in chloroform. The white crystals which deposited were filtered off and did not melt at 250°C.(482°F.). The filtrate was evaporated to dryness and a solid obtained which melted at 116 to 125°C.(240.8 to 257°F.). After two recrystallizations from acetic acid and ether, crystals were obtained melting at 133 to 135°C. (271.4 to 275°F.) corresponding to sylvestrene dibromide. The substance before bromination had given the Wallach test for sylvestrene, i.e., the acetic anhydride solution gave a deep-blue color with sulfuric acid. This is the first time sylvestrene has been found to occur in nature.

The residue after the steam distillation was a heavy fluorescent oil. It could not be concentrated further by ordinary distillation. Distillation in the molecular still gave large amounts of colorless, rancid-smelling fat which after two crystallizations from methyl alcohol melted at about 42°C.(107.6°F.). The fat probably consists largely of glycerides of palmitic and linoleic acids. Since staleness of coffee has not been proved to be connected with rancidity of the fats, the composition of this material was not studied further.

Similarly molecular distillation of the residue from steam distillation of the lower layer gave large quantities of fatty material. The steam distillate, after extraction with ether and concentration, gave an oily, phenol-containing residue. Evaporation of this gave the desired hydrocarbon, M.P. 111°C.(231.8°F.) under the microscope, but no picrate could be obtained to clinch the identification.

SUMMARY

It seems extremely improbable that changes in the flavor or aroma of coffee, known as staling, are to be wholly attributed to oxidation and consequent rancidity of the fats. The evidence indicates that the fats are not responsible for either flavor or aroma—indeed we have obtained fat-free coffee extracts in this work which possessed a rich, full coffee odor. What types of chemical reaction must occur on roasting? The present work suggests the following example:

Chlorogenic acid, which is present to about three per cent in roasted coffee, is known to lose carbon dioxide on heating and to give p-vinyl guaiacol. Distillation of this substance gives guaiacol. We have found both of these phenols in roasted coffee. Chlorogenic acid is a fairly stable substance, but p-vinyl guaiacol both oxidizes and polymerizes readily on standing. Similarly guaiacol is readily oxidized. It is unlikely that the oxidation or polymerization products are as soluble in water as the original substances. These substances —p-vinyl guaiacol and guaiacol—have powerful odors, and undoubtedly something of the complex odor of coffee must be attributed to their presence. If these compounds are destroyed by oxidation or polymerization, the resultant coffee *must* be different in taste and smell.

Another substance present in roasted coffee is furfuryl alcohol. It is a fact that this substance, when freshly distilled, is soluble in water but that on standing in air it loses this solubility rather rapidly. Thus beverage coffee, which after all is an aqueous extract, if prepared from aged roasted coffee will lack this constituent.

The terpenes, which we have shown to occur in coffee, must also be responsible for some of the taste and odor. These, too, are unstable substances tending to oxidize and polymerize on standing.

These are but a few examples of the types of changes which appear to be responsible for the alteration that time produces in the flavor and aroma of roasted coffee. Undoubtedly there are others, probably many. It is unfortunately inherent in the nature of this research that the most interesting and important substances, viewed from the standpoint of one studying the staling of coffee, are necessarily the most unstable and difficult to isolate and identify. It is not denied that the unsaturated fats are also oxidized. It is possible, indeed, that they act as oxygen carriers by way of their unstable intermediate oxidation products, and so oxidize substances of the types mentioned. These oxidations of the fats, however, are not in themselves responsible for the staleness of coffee as distinguished from the rancidity of its fats.

A careful study of the literature convinces the writers that more attention should be paid to the claims of English Patent 260.960. The work therein cited has been done by Staudinger and Reichstein, two of the best organic chemists of the present time. Staudinger is noted for his work in organic qualitative analysis and highly polymerized substances, while Reichstein first synthesized vitamin C.

Staudinger and Reichstein, working for the International Nährungs und Genussmittel-Aktiengesellschaft, have set out to prepare synthetic coffee oils. For this purpose they first analyzed natural coffee oil. They attribute the flavor and aroma of coffee not to the phenols and nitrogen compounds but "to sulfur-containing compounds of the heterocyclic mercaptan series of the general formula ASH or derivatives thereof, particularly oxy-sulfides of the formula

$$R—CH\begin{matrix}OH\\SA\end{matrix}$$

which may be obtained from carbonyl compounds, aldehydes, ketones, and diketones with the above-mentioned mercaptans by causing the compounds to react with each other."

In conclusion, the investigation has indicated the presence in the roasted coffee bean of a considerable number of substances whose sensitive nature is undoubtedly of the greatest importance in connection with the problems of aroma, flavor, and staleness of coffee. The derivatives of furane exhibit to a marked degree the tendency towards polymerization and resinification, either of which is concomitant with the loss of odor and taste characteristic of these substances in a pure state. The styrols and polyhydric phenols are prone to resinification and air oxidation alike, which processes are related to the disappearance of odor and taste, to which (in the case of closely related substances) the commercial value of many plant substances is attributed.

The terpenes surpass the other compounds not only in sensitiveness to various chemical and physical agents but also in importance where problems of flavor, aroma, or their absence, are concerned. Probably the larger portion of all plant life has as its odoriferous principle one or more of the terpenes or their derivatives. Finally, even the inactive n-heptacosane cannot be disregarded since the investigation has revealed a surprising volatility with steam, and recent research has indicated that the oxidation of normal long-chain hydrocarbons proceeds with considerable ease under conditions not inconceivable in the coffee bean.

This investigation describes the identification in roasted coffee of the following substances:

1. Kahweol
2. Diacetyl
3. Diethyl ketone
4. Vanillone
5. p-Vinyl guaiacol
6. Guaiacol
7. n-Heptacosane
8. p-Vinyl catechol
9. Sylvestrene
10. Eugenol
11. A hydrocarbon of M.P. 116 to 117° C. (111° C.).

Some of these have been previously reported in the literature and their presence confirmed by the present work. Others have not been previously mentioned.

VITAMIN C IN VEGETABLES. VII. LIMA BEANS[1]

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(Received for publication, May 1, 1937)

INTRODUCTION

Although the lima bean is an important vegetable, relatively little has been published on its vitamin C content. Fellers (1935) states that raw lima beans are an excellent source of vitamin C but does not give the amount found in the uncooked vegetable. The amount of cooked lima beans required daily to protect a guinea pig from scurvy was 3.3 grams. If we assume that .5 mg of ascorbic acid is the protective level for a guinea pig, as given by Waugh and King (1932), the cooked lima beans examined by Fellers contained about .15 mg. ascorbic acid per gram. In a later paper Fellers, Stepat, and Fitzgerald (1936) report for fresh and market lima beans, .48 and .30 mg. ascorbic acid per gram respectively. These workers also showed that during shipment in the pod in iced hampers, lima beans lose 30 per cent of their vitamin C content.

In previous work by Mack, Tressler, and King (1936) it has been shown that the variety, maturity, freshness, and sieve size of peas affect their ascorbic acid content. These same factors were considered in the investigation of the ascorbic acid content of lima beans.

In commercial practice vegetables being prepared for canning or freezing are blanched in hot water or steam until the enzymes responsible for deleterious changes in color, flavor, and aroma have been inactivated. Unpublished work on peas conducted in this laboratory has shown that a portion of the ascorbic acid is lost during blanching and that the loss becomes greater as the blanching period is prolonged. Therefore, the determination of the time of blanching which would allow the maximum retention of ascorbic acid and yet prevent enzymic spoilage in freezing storage may be of commercial significance.

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[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 197, April 8, 1937.

Publication No. 340, from the Chemistry Department, University of Pittsburgh. The investigative work at the university was made possible by a grant from The Buhl Foundation.

Presented in part before the Division of Biological Chemistry at the 93rd meeting of the American Chemical Society on April 14, 1937.

EXPERIMENTAL WORK

Method of Analysis. The modification of the Tillmans titration method described by Mack and Tressler (1937) was employed. A solution containing five per cent sulfuric acid and two per cent metaphosphoric acid was selected for the extraction process. One hundred milliliters of this acid solution extracts ascorbic acid completely from 15 grams of lima beans and prevents any appreciable oxidation of the vitamin during the analysis.

TABLE 1

Biological Assay of Vitamin C in Lima Beans

Basal diet supplement	Fed daily	Vitamin content	No. of animals	Wt. after depletion	Net gain in 21 days	Scurvy score
Lima beans, stored at	*gm.*	*mgm.*		*gm.*	*gm.*	*(0-24)*
0°C. (32°F.) then frozen...	2.5	0.5	6	329	21	4.0
Lima beans, frozen fresh...	1.8	0.5	7	330	30	3.8
Ascorbic acid solution.....	.....	0.5	9	328	12	3.9
Ascorbic acid solution.....	.....	1.0	5	348	76	1.0
None.....	.....	0.0	5	350	—132	15.0

The results of the titration method were confirmed by biological assays of two samples (Table 1). These assays were made according to the curative test used by Harris and Ray (1932). In some instances the guinea pigs would not eat the beans regularly, thus decreasing the number of animals originally started. In cases where food was not consumed within two hours, it was removed from the cages to avoid excessive oxidation of the vitamin to a physiologically inactive form.

TABLE 2

Influence of Sieve Size of Lima Beans upon Their Ascorbic Acid Content [1]

Sieve size—diameter of opening	Small-seeded varieties				Large-seeded varieties			
	Pole	Bush			Climbing or pole		Bush	
	Willow Leaved	Jackson Wonder	McCrae	Woods Prolific	King of the Garden	Challenger	Burpee	Fordhook
inch								
3/8-15/32	.48	.36	.42	.41	.61	.60	.49	.46
15/32-1/2	.41	.34	.40	.39	.63	.59	.52	.48
1/2-5/8	.39	.32	.32	.34	.51	.45	.49	.43
5/8-3/4	.....	.....	.29	.....	.40	.38	.40	.35
3/4-7/8	.....	.....	.....	.....	.34	.34	.30	.28
7/8	.....	.....	.....	.....	.31	.32	.25	.23

[1] Ascorbic acid given in milligrams per gram.

Variety and Sieve-Size Studies. Eight varieties of lima beans grown on Ontario clay loam were carefully graded according to size immediately after harvesting and then analyzed for ascorbic acid. The data presented show that in every variety the percentage of ascorbic acid decreases with increasing size of bean (Table 2). Since

in a given variety the size is approximately related to the stage of maturity, it is evident that as the beans mature their ascorbic acid content on the percentage basis decreases. Of the large-seeded varieties examined the pole types were found to be higher in vitamin C than the bush beans. Although our results indicate that a similar generalization might be made in the case of the small-seeded varieties, no definite conclusions can be drawn because some of these varieties were affected by mosaic.

If beans of any particular size are considered, e.g., those ½ to ⅝ inch in minimum diameter, it will be seen that the large-seeded varieties are much higher in ascorbic acid than the small-seeded ones. However, because of the greater proportion of small beans in the

TABLE 3

Ascorbic Acid Content of Lima Beans During Storage

Variety	Sieve size—diameter of opening	Temperature	Ascorbic acid in mg. per gm. after storage for number of days indicated			
			0	3	7	11
	inch	*°C.*				
King of Garden	⅝-¾	0	.41	.35	.32	.30
King of Garden	⅝-¾	22	.41	.30	.28	.....
McCrae	½-⅝	0	.37	.36	.33	.30
McCrae	½-⅝	22	.37	.34	.31	.18
Burpee	⅝-¾	0	.36	.31	.27	.25
Burpee	⅝-¾	22	.36	.29	.16	.....

small-seeded varieties and vice versa, the run of the pod of a small-seeded variety does not necessarily contain less vitamin C than a large-seeded variety.

Loss of Ascorbic Acid During Storage. Lima beans stored in the pod at the temperatures indicated (Table 3) retain their original ascorbic acid remarkably well for a seed-pod type of vegetable. Refrigeration retards the loss of ascorbic acid, the greatest effect being observed in the Burpee variety.

The rate of loss of ascorbic acid during storage of shelled and unshelled lima beans is recorded (Table 4). The shelled lima beans were kept in moisture-proof packages, while those in the pod were left in open containers. This procedure prevented excessive dehydration of the shelled beans, and also undoubtedly affected the rate of respiration. Under these conditions the rate of loss from the shelled beans was approximately twice that of the unshelled beans.

Although the smaller sizes of beans originally contain a greater percentage of ascorbic acid, the rate of loss is greater than it is in

the larger sizes. Hence after storage for some time, all sizes of beans contain about the same percentage of ascorbic acid (Table 5). Naturally this state of equality is reached more rapidly when the beans are stored at a higher temperature.

TABLE 4

Comparison of Rate of Loss of Ascorbic Acid in Shelled and Unshelled Burpee Lima Beans During Storage[1]

Storage time	Ascorbic acid decomposed	
	Shelled	Unshelled
days	*pct.*	*pct.*
1	11	6
2	19	10
4	29	19
7	43	25
11	58	31

[1] Original ascorbic acid content .40 mg. per gm.; sieve size 5/8-3/4 inch; temperature of storage 0°C.(32°F.).

Ascorbic Acid and Catalase Content of Blanched Lima Beans After Freezing. A number of varieties of freshly picked lima beans were graded as to size and then blanched in boiling water. The blanching period was varied from 30 to 150 seconds. After blanching, the beans were quickly cooled by immersing them in running water at 21.1°C.

TABLE 5

Effect of Sieve Size upon the Rate of Loss of Ascorbic Acid in Lima Beans During Storage

Variety	Temperature	Storage time	Ascorbic acid content in mg. per gm. of lima beans in sieve size			
			5/8-15/32 inch	15/32-1/2 inch	1/2-5/8 inch	5/8-3/4 inch
	°C.	*days*				
McCrae	....	0	.46	.42	.35	.29
McCrae	22	7	.29	.29	.30	.25
McCrae	0	19	.13	.19	.19	.17
Burpee	....	0	.49	.52	.46	.36
Burpee	0	11	.34	.42	.37	.25
King of Garden	....	0	.....	.65	.53	.41
King of Garden	22	7	.....	.34	.34	.28

(70°F.) for two minutes. They were then drained and packaged in moisture-proof, cellophane-lined, Peters-type cartons. The cartons were wrapped and sealed with waxed glassine paper. The beans were "quick frozen" in a Birdseye multiplate froster. Catalase determinations, according to the procedure of Tressler and Evers (1936), indicated that the blanching periods given (Table 6) are required to

inactivate completely the catalase in lima beans of various sizes. Peroxidase tests on these samples also gave negative results.

After four months' storage at —17.8°C. (0°F.) the various samples were cooked. All had an excellent flavor and all had a good green color except those blanched for the minimum period, i.e., 30 seconds for those passing a $^{15}/_{32}$-inch sieve and 45 seconds for those failing to pass this sieve. The color of these beans, which were apparently underblanched, had faded slightly. This seems to indicate that a somewhat longer blanching period than that required to inactivate the catalase and peroxidase should be used if a product possessing excellent color is to be obtained.

In order to determine whether or not there is any relationship between the length of blanching period and the vitamin C content of frozen lima beans, the ascorbic acid contents of three sizes of Ford-

TABLE 6

Blanching Period Required for Inactivation of Catalase and Peroxidase in Lima Beans of Various Sizes

Sieve size—diameter of opening	Blanching time
inch	*seconds*
Pass $^{15}/_{32}$	30
$^{15}/_{32}$-$^{1}/_{2}$	45
$^{1}/_{2}$-$^{5}/_{8}$	45
$^{5}/_{8}$-$^{3}/_{4}$	45
$^{3}/_{4}$-$^{7}/_{8}$	60

hook lima beans, prepared and frozen as described above, were determined (Table 7). Similar results were obtained using the King of the Garden variety.

This work shows clearly that the ascorbic acid content of the freshly frozen beans decreases as the period of blanching in boiling water is prolonged. Since the usual commercial blanching period is 150 seconds, much vitamin C could be conserved by reducing the period to 45 seconds for the small sizes and to 60 or 75 seconds for the larger sizes.

Another important factor in relation to blanching is the decreased activity of copper as an oxidative catalyst, that results from heat denaturation of vegetable proteins according to Stotz, Harrer, and King (1937).

SUMMARY AND CONCLUSIONS

Studies of the ascorbic acid content of eight varieties of green lima beans indicated definite varietal differences. When graded according to size, the large-seeded pole varieties were found to be higher

in vitamin C than the large-seeded bush varieties. When beans of a single variety are graded according to size, the smaller beans are found to contain much more ascorbic acid on a percentage basis than do the larger beans.

Lima beans slowly lose ascorbic acid during storage after harvest. The rate of loss from shelled beans is about twice that from those

TABLE 7

Effect of Blanching on Ascorbic Acid Content of Fordhook Lima Beans

Sieve size—diameter of opening	Blanching period	Catalase inactivated	Ascorbic acid content on fresh basis	Dry matter	Ascorbic acid content on dry basis	Ascorbic acid lost during preparation for freezing
inch	*seconds*	*pct.*	*mg. per gm.*	*pct.*	*mg. per gm.*	*pct.*
Pass 15/32	0	0	.46	15.0	3.1	....
Pass 15/32	30	100	.33	13.4	2.5	19
Pass 15/32	45	100	.30	12.3	2.4	23
Pass 15/32	60	100	.28	12.8	2.2	29
Pass 15/32	150	100	.24	11.3	2.1	32
1/2-5/8	0	0	.43	19.9	2.2	....
1/2-5/8	45	100	.30	17.8	1.7	23
1/2-5/8	60	100	.29	17.6	1.6	27
1/2-5/8	75	100	.28	18.4	1.5	32
1/2-5/8	150	100	.23	18.3	1.3	40
3/4-7/8	0	0	.28	27.7	1.00	....
3/4-7/8	45	92	.20	26.0	0.77	23
3/4-7/8	60	99+	.19	26.2	0.73	27
3/4-7/8	75	100	.18	25.9	0.70	30
3/4-7/8	150	100	.16	24.7	0.65	35

which are stored "in the pod." Refrigeration retards the rate of loss of ascorbic acid during storage.

Approximately one-third of the ascorbic acid of lima beans is lost during blanching according to the usual process followed in preparing the beans for freezing. This loss can be materially reduced by cutting the blanching time in half. The shorter blanching time was found to be adequate for inactivation of the enzymes which cause the development of off-flavors and the loss of color in the frozen product during storage.

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EDITORIAL REVIEW

FOOD FLAVORS—A CRITICAL REVIEW OF RECENT LITERATURE

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(Received for publication, June 30, 1937)

The flavors of foods and beverages are of the greatest human interest and practical importance. A new approach to the subject of flavor consists in attacking several of its many sides simultaneously, but especially the psychological and the chemical sides. The series of papers gathered in this periodical is a coördinated attempt to survey the field of flavor and to outline some of its more important problems.

The physiologist and the psychologist have determined experimentally that food must be attractive to be eaten in quantity and to be digested properly. Pavlov (1902) showed by careful experiments on dogs that pleasant anticipation, quite as well as realization, caused digestive juices to flow; and conversely that fear or unpleasantness retarded or inhibited their appearance. In a most charming book, Brillat-Savarin (1838) developed a complete philosophy of dining and concluded that a smile or a good story could be a real appetizer. Even more forcefully effective is appropriate aroma and good appearance of the food set before one, and the texture and taste as it is being eaten.

Sternberg (1914) pointed out that one naturally eats and swallows pleasant-tasting foods rapidly, rather than lets them linger in the mouth. If one holds food long in the mouth, it is an indication that the taste is not entirely a pleasant one. He characterized the existence of Fletcherizing as due to lack of appreciation of the physiology of taste.

Good flavor is to many people an evidence of high quality. While many people are partially smell-blind or taste-blind, as Fox (1932) and Blakeslee (1935) have found, there are comparatively few to whom food flavor has not a positive and forceful appeal. Free (1926) showed that most people have really good perception of the odor part of flavor but are quite uneducated in correlating or describing their sensations. A relatively few people have educated senses with the ability to describe their sensations as objective realities. The influence of these latter people is great, as pointed out by Moir (1936),

whether as writers, chefs, or friends; for many others look to the discriminating few for advice and opinion in this as in many other departments of human interest.

In any event, flavor is such an important part of a food or beverage that it cannot be neglected with impunity by any producer or distributor. As improved kinds of foods appear, and as improved packaging, handling, and processing produce foods of better appearance and variety, attention to flavor becomes increasingly important.

Thorbjörnson (1936) emphasizes that single or simple flavorings usually do not satisfy for a long period because the senses soon recognize their simplicity and lose interest in them. This is further developed in Smith's paper, elsewhere in this periodical, on the need of blended and otherwise developed flavorings.

Again, the public takes it for granted that variety should come in flavor. This is perhaps not so much because the public is fickle as that it is becoming educated, and with education comes appreciation and demand for more and better things. "Streamlining" may apply quite as much to the styling and flavor improvement of a line of foods as it does to the unification of line of an automobile.

Finding out what the public likes, or will like when presented, is a necessary part of the cycle of flavor development. How this may be done is discussed in three papers of this series as applying to different kinds of workers. This sampling of the public taste is so very important, especially before a big outlay is risked in manufacturing and sales promotion, that some service organizations now exist primarily to handle this work in a systematic manner.

Since flavoring materials are chemical substances, whether simple or complicated mixtures and whether made in the laboratory of nature or that of man, there naturally exist many opportunities for the services of the chemist to see that these chemicals are treated properly.

SENSES USED IN DETECTING FLAVOR

Flavor is the part of food and drink that affects our senses of taste and smell. Practical control over flavor has long been an art and probably will remain an art. There is need today, however, for the application of science to the flavoring art in order to advance it to a higher status where it will become more exact and more generally useful. Many food problems have been kept from solution for lack of sufficient exact information about flavor. That condition is in the process of correction.

The physiology of the senses connected with eating quality is most fully described by Von Schramlik (1926) and in English by Parker (1922).

The sense of taste operates through taste buds located principally on the tongue. Taylor (1928) has proposed a physicochemical explanation of tasting. Most experimenters today are satisfied that there are but four kinds of taste buds, each corresponding to a distinct kind of taste element of which the accepted types are sweet, sour, salty, and bitter. The relation between taste and chemical constitution is described by Cohn (1914, 1916).

The sense of smell, closely associated in the popular mind with taste, operates through free nerve-ends rather than through "buds" in an inaccessible area smaller than a postage stamp high in the nasal cavity back of the eyes. Physiologists have not yet succeeded in distinguishing any definite number of nerve types. This makes it necessary, at least for the present, to classify odors indirectly by the sensations produced.

Many systems of odor classification based on sensation have been suggested. Henning (1921, 1924) points out the gradations between tastes and those between odors. He depicts the four primary tastes graphically as the corners of a regular tetrahedron, indicating the possible tastes intermediate between the corners. Similarly he shows six primary odors—flowery, fruity, foul, spicy, resinous, and burnt—which he here depicts graphically as the corners of a triangular prism. Henning's book, "Der Geruch," is one of the fundamental references on this subject.

Zwaardemaker's classification of 1914, discussed by Bogert (1922), includes nine classes with many of these divided into sub-classes. It is evident that, while this may be a convenient system of classification, it is hardly to be expected that there are this many primary or basic odors corresponding to the relatively few primary colors and tastes. Zwaardemaker's main classes are as follows: ethereal, aromatic, fragrant or balsamic, ambrosial (as musk), alliaceous (as garlic and many compounds of sulphur), empyreumatic or burnt, caprylic, pyridin, indol (suggestive of putrefaction or feces).

Attempts to connect chemical constitution with odor have been made by many, including Bogert (1929), Michael (1932), Mee (1934), and Hewitt (1937). These reviews are valuable in focusing attention on the active chemical groupings responsible for the respective stimulations. In addition to quality and quantity, Michael attempts to establish a third characteristic of odors which he describes as "brightness" (Helligkeit). Dyson (1937) has an interesting paper showing a definite relationship between odor and the Raman shift. This is used to form a classification of odors according to the extent of the Raman shift, just as colors may be classified to wave lengths of light.

Kenneth (1922-4) gives a bibliography of one thousand items on smell and Zwaardemaker (1926), in the International Critical Tables, outlines an attempt at a scientific system of odorimetry. The threshold values of many common substances are stated, based upon carefully standardized conditions. A number of physical constants are given with references to the original authorities.

Crocker and others have pointed out the importance of vapor pressure in odor blends. Tammann and Oelsen (1928) go even further and claim that the vapor pressure of an odor can be calculated from the threshold value within certain limitations. Kenneth (1927) has also studied odors from the psychological approach, experimenting with their pleasantness or unpleasantness and their associations (technically affects) in the minds of the subjects. However, the classification of Crocker and Henderson (1927), further described by them (1934), is the simplest and seems perhaps best adapted for use on great numbers and varieties of odor types. The fundamental sensations defined by this system are fragrant, acid, burnt, and caprylic.

The degree to which a given odor contains each of these fundamental sensations is indicated by nine numbers running from zero to eight inclusive. Thus, the odor of rose is represented by 6423, indicating six degrees of fragrance, four degrees of acidity, two degrees of burnt sensation, and three degrees of caprylic, which is the disagreeable component suggesting putridity. By contrast, the odor of acetic acid is represented by 3803. A critical study of this method was made by Boring (1928) who found excellent reproducibility by independent observers. Boring concludes from this study that from 2,000 to 4,000 different odors can be distinguished from one another by the average individual. This system has been given practical application.

There are nerves of the sense of feeling in the mouth, through which a part of the flavor of foods and all of texture are perceived. The generally recognized feeling components are warmth, coolness, contact, and pain.

Physical tests as directly related to food products were made by Platt and Fleming (1923), Platt (1930), and Platt and Kratz (1933). For many practical applications, such as vegetable cooking, the first consideration may be tenderness to penetration by a fork. Sweetman (1931) stresses the importance of this factor. Sandreth (1929) reviewed some of the pieces of apparatus which may be used in testing the tenderness of sweet corn, carrots, and other vegetables. Tressler, Birdseye, and Murray (1932) devised an apparatus for testing the tenderness of cooked meats variously treated, especially by freezing. Many other sensations, such as the amount of saliva necessary for

swallowing, according to Whymper (1919), may be included under the general term of "mouth feel." This is perhaps the least studied component of "eating quality." Sherman (1931) cautions against being guided too much by eye and the sense of touch in judging foods to the exclusion of flavor factors.

The senses of taste and feeling have long been favorite subjects for investigation by physiologists, and much exact information is available concerning the distribution of the several kinds of nerve-ends or sensitive organs involved in their detection. During the past few years the physiologist has had the benefit of vacuum-tube amplifiers and other observation-extending instruments of the physicist, by means of which he has studied the nature of the electrical factor of nerve response and stimulus transmission to the brain. The sense of smell is so situated, however, as to be difficult to work with in the functioning organism, and knowledge of its physiology consequently has lagged behind that of taste and feeling.

All workers agree that flavor involves simultaneous tasting and smelling, with the tasting being done in the mouth and the smelling in the nose from the vapors that come up from the mouth by way of the back of the throat. Some workers consider that the feeling factors in the mouth also are part of flavor, especially any feeling of pungency, coolness, or greasiness of the food. Whether this should even take in graininess or the gross texture of the food may have to be decided upon by usage of the term *flavor* over a period of time. At any rate, flavor is a complex of sensations and must be so treated.

FLAVOR STIMULI

There is no dearth of information about actual stimulants for taste and smell, and to some extent, for feeling. The perfumer, besides having a wealth of extracts of flowers, leaves, roots, and animal products, has had the services of the organic chemist at his command for several generations to produce useful smell stimuli to match those of nature and to cover new territory. The food worker is more limited in his choice, for his stimuli must not only be effective but must be harmless when consumed. In general, they must be those of the foods themselves and a few other products of natural origin, such as sugars, acids, and spices. In bread, cereals, maple syrup, tobacco, coffee, and some other processed goods heat, acting on natural materials, produces developed flavors often of delicacy and great interest. Fermentation is also a permissible flavor-building operation for both foods and beverages.

Not much of novelty has entered the field of taste stimulants of late. The old standard stimuli for sweet, sour, salty, and bitter are

very generally used in most food products. Occasionally a new sugar or a new acid appears on the market and is of interest for some specific reason, such as price, chemical stability, or for dietary reasons. Those synthetic taste stimuli that have been introduced have been principally sugar substitutes, such as saccharine.

Attempts have been made by Crocker and Henderson (1932) and Baryscheva (1926) to duplicate tastes in terms of the taste fundamentals. Duplications of odor sensations have been many times attempted but with less success; for, as stated by Thorbjörnson (1936) and as every perfumer realizes, the sense of smell is extremely analytical and the higher centers do not coördinate the received impressions. If there are perhaps three or four individual chemicals in admixture, one's nose usually tells him that it is a mixture; and if trained enough, it may even be able to identify most of the individual substances. Exceptions are true blends which are mixtures of substances of the same vapor pressure in rough "threshold" proportions. Mixtures have been duplicated by other mixtures containing some different ingredients, but these constitute notable exceptions to the rule and are rated as triumphs by the perfumers who achieved the results.

A study on the covering-up of unpleasant tastes or odors, especially in medicine, was reported by Wright (1937). He found that cocoa and raspberry are the best all-round agents for disguising bad flavors; but cinnamon, orange, or sarsaparilla are better for hiding specifically salty tastes.

THE LANGUAGE OF FLAVOR

The status of flavor language is low, especially for the odor constituents. The lack of a generally accepted odor classification system has held back the development of adequate and accurate language. This was discussed by Crocker (1935) with suggestions for team work between various groups of scientists to correct the situation.

At the present time most odors are described as like certain others with which the reader is presumably more familiar. This is fairly effective in the hands of the professional perfumer who has a rich background of familiarity with the odoriferous products produced by nature or man, but even he asks for something better. With people who are less trained, both observation and background are apt to be faulty and description weak and very unsatisfactory. Lack of proper words to express ideas is evidently a serious handicap in scientific progress.

In a study of the taste-names of primitive peoples of the present day and of civilized people of several generations ago, Myers (1904)

found that the language is usually extremely faulty. Salty and sour are often confused. Bitter may be only unpleasant and salt may be sweet, alluding doubtless to its ability to make foods more palatable. In the more developed races, however, even several thousand years ago, some attempt appeared at definiteness of taste description, as shown by the six tastes of the Hindoos and the nine tastes of the Greeks (sweet, salty, sour, bitter, astringent, dry, pungent, vinous, and oily).

CONSUMER PREFERENCE TESTS BY THE GENERAL PUBLIC

Organoleptic tests on foods may be sharply divided into tests for the *amount* of some quality and tests for *preference* for some quality. Tests for amount include determination of threshold sensations, and estimations as to which of several samples is strongest in a given flavor. Questions as to preference, however, introduce several entirely different factors. Failure to recognize this is the cause of some of the confusion which exists in this field.

Preferences for foods originate from many causes, prominent among which is custom. Remington (1936) surveys food habits in different parts of the world and in different ages. His paper on "Social Origins of Dietary Habits" states, "dietary instinct is a distrust for the unknown, for those things of unfamiliar taste, and nothing more." He shows the illogical nature of many food customs. In individuals, as in the race, preferences arise from custom and frequently from pleasant or unpleasant associations reaching back even to childhood.

Some of the essentials in scoring food products, including the importance of imagination and suggestion, have been described by Platt (1931). When limited numbers of more or less trained and controlled judges can be used, much may be done in testing the ability of the individual judges, as related by King in an accompanying paper on food flavors. Results satisfactory both as to uniformity and reliability can usually be obtained.

When an attempt is made to determine the food preferences of the general public, many additional complications ensue. These are due partly to the fact that the public (even in one city) is made up of groups which differ widely in race, nationality, and income class. Such differences are often reflected in different food habits and hence in different food preferences. In the face of these differences the method of selecting the sample of the population concerned is evidently of prime importance. In this and in estimating the significance of differences, the ordinary methods of statistical analysis are often helpful and in some cases essential.

Studies of sections of the general public to determine their habits and preferences are becoming more and more common. These may have a scientific purpose, as shown in sociological studies by Fry (1934), or a commercial purpose, in which case such activity is often included under the name of "market research." Some phases of market research have been well developed. References to its general aspects or to market research on foods, which is concerned merely with the collection of statistics of consumption and distribution without attention to consumer preferences, are beyond the scope of this review. "Standards of Research" by Carroll (1929) will be found helpful in maintaining standards of accuracy and impartiality in a field where there is often a temptation towards bias. That part of market research pertaining directly to the determination of preferences for foods has been relatively neglected. A very recent book prepared by the Committee on Marketing Research Technique of the American Marketing Society (1937) is an unusually comprehensive treatise on this subject and contains much of interest on the general aspects of market research, yet the special technique necessary for judging foods is hardly mentioned either in the text or in the copious bibliography. This is some indication of the scarcity of published material of real merit on this subject. Another excellent critical bibliography on the general phases of marketing research, but again with little that is specific for food preferences, is that by Kelsey (1935).

In a popular article Platt (1937) has described one method for the evaluation of consumer preferences for food products. Some of the more theoretical aspects of this broad subject are covered by the same author in the symposium following this review.

One of the most comprehensive investigations of public preferences for foods is described by Hobson and Schaars (1935). This article is well worth careful study both for its achievements and for the difficulties which it discloses, chief among which is the considerable expense involved. This investigation is a striking illustration of the important, but often overlooked, fact that in the present development of technique no one method for the determination of consumer preferences is quite adequate to tell the whole story. It is sometimes necessary and frequently helpful to attack the same problem by several different methods and then to compare results obtained. In the study by Hobson and Schaars, for example, public preferences for different grades of cheese were determined by the usual method of asking for preferences between two samples from customers in city stores and from farm people at an agricultural convention. It included also methods by which selected groups were allowed to eat all of several

different types of cheese that they desired for periods of from one to fifteen weeks. This latter method brings in the time factor in consumer preferences, which is often neglected in such tests but which is demonstrated to be of importance. An earlier investigation of a somewhat similar problem was conducted by Waugh (1929). This was a critical study of factors which influence demand in the purchase of vegetables by the general public. Difficulties were not glossed over.

Other descriptions of competent investigations going direct to the general public are "Consumer Preferences for Egg Yolk Color and Shell Color in New York City" by Thomsen and Winton (1933), using the method of store sampling; and "A Study of Consumers' Preferences and Practices in Buying and Using Eggs" by Corbett (1933), using direct interviews. Link, et al (1935) provide another example of a study going direct to the general public to determine the many factors which influence the purchase and use of a food product. Full details are given. It points out the many so-called psychological factors which may influence the use of a food beyond those directly connected with taste, odor, and mouth feel. A closely related consumer preference study is that of Brown and Lazarfeld (1936). Both of these latter are interesting from the standpoint of methods.

If two or more samples of food are studied in order to determine public preference, we may conclude from our studies that Sample A is preferred over Sample B; or we may conclude that there is no significant difference in preference between A and B. Either of these conclusions may be correct and if so, is satisfactory and is a positive contribution to our knowledge. Very frequently in such tests there is, in fact, no real difference in the public preference between the two samples. It would be a serious mistake to indicate a preference if none existed.

Occasionally after a preference test has been completed, it is not possible for the authors to state whether or not any definite preference exists. Several articles containing such inconclusive results have been published recently. With the modern technique for consumer preference tests it should now be possible to frame the method and scope of the test so that some definite conclusion would be forthcoming. Such a conclusion might be stated with certain clearly defined limitations but should be none the less definite within these limits.

THE ART OF PROFESSIONAL TASTERS

There are tasters who have been trained or have disciplined themselves to the point that they smell and taste commercial products in a dependable and reproducible manner. Several tea-tasters or several

coffee-cuppers will check one another's findings, or repeat their own, with remarkable accuracy. These men operate systematically, with plenty of checks and against standard samples, to keep themselves on the track. Testing is done in series of one to two dozen cups each, and the judging is more comparative than absolute. Only a single factor is tasted for at a time. Usually smelling is done first, for rough-grading, and then mouth-tasting is done to check these findings and to discover taste factors in addition to the smell factors. Maiden (1936) points out that bakers judge bread flavor almost entirely by smell, whereas the consumer has not learned that easier method and has to taste it.

Tasters of other products, such as tobacco, wine, dairy articles, baked goods, mayonnaise, and a host of other food products, grade these articles by scoring systems that are quite widely acceptable in buying and selling. Some of these systems are illogical but are justified by their wide acceptance.

Chefs are highly trained chief cooks who may become executives of one type or another; for example, "Oscar of the Waldorf" is a host and George B. Rector, an advertising counselor. On rare occasions a chef may invent some new dish, but most cooking is conservative and repetitive, and most flavor matters are handled by the actual cooks. Formulas and recipes usually are followed religiously, and even "season to taste" tends to have well-standardized meaning.

In working with professional tasters the feeling grows on one that these men do their work exceedingly well. They are mostly not geniuses or more sensitive in detection than many other people, but they are workers in a field where the systematic application of senses is highly effective.

SOME CHEMICAL PROBLEMS OF FLAVOR

The influence of containers and of air, heat, light, and other chemical factors on the flavor of canned and bottled foods and beverages has been studied, especially by the producers of containers. Their findings are rarely published, but when a victory is finally won over some specific problem, there appears on the market a new product or an old product in a new container, such as beer in cans or sausages in Cellophane. In this class comes the highly important industry of frozen foods, where flavor is maintained by the combined actions of very low temperature, solid form, and exclusion of oxygen and other deteriorating influences.

Much attention is being given to shortenings and to baked goods containing them, as well as to natural fatty foods and meats, to prevent the development of very objectionable fatty rancidity. Newton (1936) has reviewed extensively progress made in this field. Punnett,

Eddy, and McNulty (1937) give information on the rancidity of coffee and tests for rancid coffee.

Unpleasant odors may be removed from watery or oily liquids by treatment with activated solids, frequently carbon. Many temporarily or permanently poor water supplies have the water made palatable by the use of an auxiliary treatment with activated carbon, and this type of treatment has received the benefit of much chemical engineering experience, most of which fortunately has been published. Standardized technique and apparatus are now available for judging the quality of raw and treated waters. Carbon is said also to be usable for purifying gelatin, not only for odor and taste but also for color and turbidity, by treatment while it is in solution form. Fats and oils may be deodorized and also otherwise improved by similar physical treatment with or without the use of steam, vacuum, or chemicals.

CONCLUSION

The study of foods may be thought of primarily as a popular subject and only secondarily as scientific. This is justified, although few realize to what a large extent science—especially the sciences of chemistry, bacteriology, and physiology—has invaded this largest and most fundamental of all industries. In the recent past most of the discoveries regarding the healthfulness, production, and storage of foods have originated with the scientist, and this will probably be true to an ever increasing extent in the future.

Being considered a popular subject, it is not surprising to find a large amount of semi-popular literature on the subject of food flavors. Much of this is of little scientific value, and some of it is based upon assumptions which are not well established.

Food flavors and the perfection of methods for the determination of human preferences for foods are subjects touching on many sciences and are replete with difficulties. That our present knowledge is sketchy should be frankly recognized; however, much progress has already been made as will be seen by the literature herein mentioned. More progress will be made by more and more quantitative studies, each based upon careful experiment and planned according to the best traditions of the scientific method.

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THE FLAVOR OF MEAT AND MEAT PRODUCTS[1]

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(Received for publication, May 22, 1937)

Meat flavors may be defined as those qualities in meat that affect the taste. They are the result of the action of stimuli, chemical in nature, on the various taste buds of the tongue. Depending on the concentration and intensity of the various stimuli a greater or fewer number of the taste buds will be acted upon, hence the general response obtained from different substances will vary. In meat there are several flavoring substances that may be recognized with the added possibility of new compounds formed during cooking.

The United States Department of Agriculture, in coöperation with various state experiment stations, has been engaged for over ten years in a study of the quality of meat and the factors that modify it. The complex problem of flavor has been considered in a subjective manner; little has been accomplished objectively. It is necessary, therefore, to try to bring together facts that have been developed by observation and a few experimental results.

VARIOUS QUALITIES CONTRIBUTING TO FLAVOR

Before discussing the factors affecting flavor in meat, reference should be made to certain other factors often considered as contributing to flavor, although they may not belong strictly under such a heading. Two such factors are "smoothness," or the lubrication of the lipids, which contributes to a quality that might be designated as "richness" of the meat and to the quantity of juice. It is possible that some persons associate tenderness, or the degree of resistance to pressure by the mouth and teeth, and texture of meat as factors in flavor. The appearance of meat also influences its flavor for some persons. It is obvious, then, that the flavor of meat should be recognized as a combination of a number of variable factors which undoubtedly leave different impressions with different persons but which each recognizes as a meat flavor.

Meat flavor, however, in its truest sense, consists of the stimuli given to the taste buds by the inherent organic and inorganic substances, such as water soluble extractives, lipids, small amounts of

[1] Read before a symposium on *Flavors in Foods* at the Chapel Hill, North Carolina, meeting of the American Chemical Society, April 12 to 15, 1937.

carbohydrates and salts, or compounds produced from these products and proteins by cooking, by enzyme action, or by both.

DETERMINING INHERENT DIFFERENCES IN FLAVOR

In planning studies to determine the inherent differences in the flavor of fat and lean—the differences owing to factors of breed, feed, or management—care must be taken to avoid or standardize the effects of cooking or enzymes or other factors on meat flavor and to include samples of meat that are as similar as possible.

The development of meat flavors has been described by Grindley and Emmett (1905)[2] as due very largely to cleavage of extractive bodies in the meat juice and to cleavage of the fat under the influence of heat. Rare cooked meat has been found least affected by heat. The flavors, therefore, consist of the natural unchanged or slightly changed flavor of the fats and extractives. In beef, the meat most often cooked only to the rare stage, there is an acid taste probably from lactic acid, phosphates, etc. Such acidity is most often observed in beef from young cattle. This observation does not agree, however, with the alkaline taste described by Grindley and Emmett from meat juice heated to 65°C.(149°F.). At 65°C., according to Grindley and Emmett, an unpleasant alkaline flavor develops in the juice, which is not modified by continued cooking at 65 to 100°C.(149 to 212°F.). Changes produced in the dried juice or soluble part of the meat by temperatures over 100°C. are in a large measure responsible for the flavor of meat cooked by dry heat. A characteristic flavor is produced in fatty tissue which becomes more pronounced at higher temperatures, especially where browning occurs. These changes in fat and other substances are apparently due to cleavage produced by heat. Beyond these general facts little is known of the nature of the chemical compounds formed.

Studies on the quality of meat have been planned by the U. S. Department of Agriculture especially to determine whether or not there are differences in the inherent flavor of the lean and fat. The method of handling meat and the length of preliminary storage or ripening period of dressed meat have been carefully standardized. Cooking, also, has been largely restricted to the medium rare stage. In carving, care is taken that the sample to be judged is not contaminated by the developed flavor from the browned outer surface or extra fat. The lean and fat samples are taken from the interior of the roast.

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[2] Grindley, H. S., and Emmett, A. D., 1905. Studies on the influence of cooking upon the nutritive value of meats at the University of Illinois, 1903-1904. U. S. Dept, Agr., Office of Experiment Stations, Bul. 162.

The palatability studies are conducted by a trained group of judges who pass on both desirability and intensity of flavor in addition to other characteristics of meat. Both fat and lean are judged warm and unsalted. Odors are considered briefly under the terms of intensity and desirability of aroma. Roasted or broiled meats have two zones of flavor—the outer portion that has been subjected to high heat and the inner portion that has been heated slowly and chiefly by conduction. Inner flavors are considered to be due to the salts, lactic acid, etc.; outer flavors are those due to the cleavage products produced by roasting or cooking.

During roasting, boiling, or cooking by other methods very small losses of nitrogen and other non-nitrogenous compounds take place into the air. Part of the water and the solids lost from cooked meat appear in the gravy. The housewife makes use of this rich supply of flavoring agents and applies additional heat for desirable developed flavor in the preparation of brown gravies. The material used is the browned meat juices that have exuded from the meat during cooking. Negative evidence of the effect of heat is found in the gravy from meat cooked at low oven temperatures which does not have as strong a meat flavor as that from meats cooked at higher temperatures. These developed flavors of meat are supplemented by the dextrinization of the added starch.

It is often said, but not confirmed, that the addition of salt to meat develops the flavor. No work has been reported on the subject. A few experiments have been done by the U. S. Department of Agriculture in adding salt to the samples of meat to be judged in the regular way. It is our opinion that the addition of salt does not increase the inherent meat flavor but sets up new stimuli owing to the salt. This new combination is the result of the enhanced salty flavor. After working with unsalted meat for a period of time the addition of salt often ceases to be necessary for complete satisfaction in eating meat.

An interesting observation was made in some preliminary tests on different kinds of meat by people who were blindfolded. Of the better known meats used in these tests it was found that beef and pork were more intense in flavor and more often correctly identified than chicken, veal, lamb, or rabbit. While there may be some question about the ability of blindfolded judges to distinguish between lean portions of different kinds of meat that had not been exposed to high heat, the outer portions with the modified extractives and fat were more easily distinguished. In this case it is probable that the characteristics lie chiefly in a specific constituent of the kind of fat.

FLAVOR DEVELOPED BY AGING OF MEAT

An interesting example of developed flavor is the southern ham. After the ham has been cured and smoked, it is allowed to age for varying lengths of time up to a year or more. During this time it becomes partially dry. The characteristics of such hams are a sharp, cheesy flavor in the lean and a modification of the fat so that it has a slightly rancid taste. This change in flavor is accompanied by a clearing of the fat so that it becomes semitransparent. Clusters of tyrosine crystals are often found in the lean meat. The University of Maryland [3] has been able to produce certain of the characteristic flavors in ham in a relatively short time, six to ten weeks, by incubating cured and smoked hams at 42 to 52°C.(107.6 to 125.6°F.). Hams cooked after eight weeks of aging did not show as pronounced a cheesy or spicy aroma as those cooked at the end of ten weeks. Intensity of aroma increased with aging. The flavor of incubator hams was more pleasing than the strikingly different freshly smoked commercial hams.

The chemical study of "quick aging" of hams shows the following changes in the meat:

(a) Increase in the free fatty acids of both meat fat and adipose tissue fat. (b) Hydrolysis of the protein, resulting in an increase in total soluble amino nitrogen. (c) Loss of moisture from the lean tissues. The changes are characteristic of the aging process occurring in hams.

Off or foreign odors and flavors in meat may occur from several causes. They may be noticeable when the animals have eaten cod-liver oil, fenugreek, mint, wild celery, anise oil, or certain chemicals; also when meat is stored in a refrigerator in close contact with certain foods and fruits it often absorbs foreign odors. Occasionally the meat from very old animals, such as bulls, cows, or rams, develops an extremely unpleasant odor during cooking, often characterized as ammoniacal. Sexual odors in tissues are usually associated with boars and with stags that have been slaughtered soon after castration. Little or none of this kind of meat is found in commercial channels. Off-flavors are found occasionally in the meat from apparently normal animals. No explanation for this condition has been discovered.

MEAT EXTRACTS USED TO IMPART FLAVOR

Meat flavors are often extended or added to other foods through the use of commercially made extracts or juices. These products are

[3] Hunt, W. E., May, 1935. Secret of old ham. The Country Gentleman 105, 18.

made by separating the nitrogenous extractives from fresh meat with water, removing the fat, and concentrating the liquid portion by evaporation.

Such an extract contains not less than 75 per cent of total solids, of which not over 27 per cent is ash, not over 12 per cent is NaCl, not over .6 per cent is fat, and not less than 8 per cent is nitrogen. The nitrogenous compounds contain not less than 40 per cent of meat bases and not less than 10 per cent of creatine and creatinine.

Meat extracts are also prepared which are concentrated at a lower temperature than the extract of Liebig type, such as that produced by Valentine. Such extracts have less of the developed flavor and are distinctly acid. They contain between 50 and 75 per cent solids. In this case a large proportion of the creatine is removed.

Yeast extracts and vegetable protein digests are used in flavoring agents to be employed as substitutes for meat flavors. Yeast extracts contain no creatine or creatinine. The distribution of the various xanthine bases is different in meat and yeast extracts. In meat extracts xanthine and hypoxanthine predominate, whereas in yeast extracts adenine and guanine predominate.

The Orientals have established an extensive literature on hydrolyzed proteins, mostly of vegetable and fish sources. It is their belief that the glutamic acid, which is an important constituent of these hydrolyzed proteins, is the constituent responsible for meat flavor. Monosodium glutamate is also recommended by them for use as a meat substitute. This conclusion has not been our experience. Several commercial products used as meat flavoring substitutes have been purified and recrystallized many times. This recrystallized material has been offered to judges in solutions of high molarity and also in dry form, and meat flavor has not been associated with them. It is our belief that this alleged meat flavor, if present in the original compound, must be due to the impurities present rather than to glutamic acid or its non-sodium salt.

SUMMARY

There is little concrete evidence as to the chemical characteristics of meat flavor. The responsible constituents, as ordinarily recognized, appear to consist of a composite of salts, acids, and a group of products resulting from heating extractives, possibly disintegration products of proteins, and lipids. The quantity and quality of these products are related to the extent and duration of heating. There are certain inherent flavors in the fat of meat, that are characteristic of species, which become evident when the fat is heated. Fats contribute body, richness, or smoothness to meat juices or gravies which,

although chiefly mechanical in effect, have much to do with the desirability of meat. Age of the animal also has an important influence on flavor of the meat, such as baby beef in contrast to beef from more mature cattle and lamb in contrast to mutton.

Ripening of meat results in an increase in soluble protein products which, when heated, play an important part in the flavor of such meats. New flavors are developed by aging of cured meats, characterized by the southern ham. The new flavors are apparently disintegration products of protein and fat characterized by such terms as cheesy, pungent, and spicy.

USE OF ACTIVATED CARBON FOR CORRECTION OF FLAVORS[1]

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(Received for publication, May 22, 1937)

INTRODUCTION

We are apt to feel that we have become conscious of having the proper flavor in our food products, although in many cases we are still using the age-old method of masking an unpleasant flavor with spices and flavoring extracts. Probably because of education and capable advertising, esthetic attitudes have changed. Most foods we dislike to think of having dressed up with spices and we prefer a so-called neutral flavor. Unconsciously we feel that this neutral flavor indicates greater purity and cleaner manufacturing conditions. A neutral flavor is desirable in the raw material even when flavoring extracts are to be later added, for then the resulting flavor can be of a delicate character. Biological control, including such things as sanitary manufacturing conditions and refrigeration, have been of tremendous value; but in spite of the best efforts a final product may at times have an off-flavor. This may be due to slips in the control or to the type of raw material employed. When this happens, some means for correcting the condition, such as a chemical treatment or use of steam for deodorization, can be used. Unfortunately, these methods have the disadvantage that at times they may produce an unfavorable reaction in the product itself.

ACTIVATED CARBON AS AN ADSORPTION AGENT

A corrective method that is meeting with increasing favor is the use of an adsorption medium which removes the undesirable flavor but leaves the product in a pure and unchanged form. Activated charcoal, or activated carbon, is one of the most useful adsorption agents. As a result of its successful use in improving the palatability of municipal water supplies, it has come into considerable prominence in recent years.

Activated carbon, as found on the market, is a black powder closely resembling ground charcoal. Today it is realized that the term "activated carbon" does not refer to a single simple substance as in the

[1]Read before a symposium on *Flavors in Foods* at the Chapel Hill, North Carolina, meeting of the American Chemical Society, April 12 to 15, 1937.

case of copper sulfate or calcium carbonate, but the term refers to a class of products. The specific adsorption properties of activated carbon depend largely on the method of manufacture. A carbon made by one process may be quite effective in decolorizing sugar solutions, but this same quality of carbon may be relatively ineffective for removing off-flavors from water supplies. On the other hand, a carbon made by a different process may have these properties in reverse order.

Colloid chemistry has frequently been properly called "surface chemistry" and it is through a study of this subject that the explanation for the removal of tastes and odors by activated carbon can be found. Activated carbon, when suspended in a liquid, provides a large interfacial surface. Willard Gibbs (1876) stated that if a dissolved body lowered the surface tension of the liquid in which it was present, then this body would tend to move to any surface or interface and become concentrated there. It so happens that many taste and odor bodies lower the surface tension of liquids. When activated carbon is introduced into such a liquid, these taste-bearing bodies move to the interfacial surface, that is, at the carbon and liquid boundary.

While this explains the mechanics of providing the original contact of the carbon and taste body, it does not explain why other porous bodies are so much less effective or why different carbons will vary widely in their selective adsorption of particular tastes, odors, or colors. Unfortunately, there is no adequate, simple explanation of this. Often it is interpreted as being due to the free valences which hold these bodies, once a contact has been established.

It will be apparent that the use of activated carbon is limited to those products which can be reduced to a liquid or fluid condition for treatment. It is also limited to tastes or odors owing to the presence of foreign bodies; for example, it is not possible to make quinine less bitter by treating with activated carbon, inasmuch as bitterness is a physical property of quinine. If a liquid happens to contain quinine, however, then activated carbon will remove the bitter taste by actually adsorbing the quinine and removing it from the solution.

TWO CLASSES OF TASTE AND ODOR PRODUCTS

Taste and odor problems subject to removal by activated carbon can be put into two classes. One is that in which complete removal of foreign taste and odor is desired, such as in the case of lard, gelatin, or sugar. This is a matter of selecting the carbon that will do the work most effectively at the lowest cost. In brief, the manner of determining this is to mix up various weights of activated carbon

with the liquid in question at various temperatures and for various periods of time, then filtering and judging the filtrate for improvement in flavor. The successful use of activated carbon in removing tastes and odors from varied water supplies where practically every conceivable taste has been found, will indicate how universal is the value of activated carbon for removing tastes and odors.

The other group comprises problems of removing an objectionable odor without affecting certain other desirable flavors. This is usually much more difficult, but owing to the selective action of different carbons for different flavors, it can often be solved. Frequently it involves special methods of application, as in the treatment of alcoholic liquors, including spoiled wines. Here it is sometimes necessary to remove objectionable odors without at the same time destroying the bouquet. It is known that the use of certain special activated carbons, introduced in small quantities of less than .01 per cent and kept in contact for a month or more, will produce the desired result.

While it has just been mentioned that a month or more is required in some special cases, for most purposes it will generally be found that the action of activated carbon is very rapid. Usually less than an hour is sufficient.

The best temperature to use can usually be determined only by experience. Higher temperatures reduce the viscosity of solutions and for that reason increase the ease of filtration. It is, however, always important to keep the temperature below the point where the product might suffer any deterioration.

PURIFICATION OF VARIOUS FOOD PRODUCTS

Activated carbon has been extensively used for years in the purification of many products, and it might be well to give details of several instances briefly. Of these products gelatin is one of great importance. This is one of our finest foodstuffs because of its very high protein content and because of the availability of the protein which it contains. The name "gelatin" probably brings to mind those amazingly beautiful, transparent, molded desserts which are either colorless or have a vividly live fruit color. Activated carbon plays an important part in producing and maintaining this sparkling brilliancy, but of equal importance is the perfect elimination of all traces of taste and odor originating from the raw materials from which gelatin is produced. Naturally traces of animal odor may be present in even the highest grade of fresh skins, bones, and other gelatin-bearing parts; and it is the regular and consistent use of the proper grade of activated carbon that insures production of odor-free gelatin of uniformly neutral taste. A ten-per cent solution of gelatin

and water has for the most part been found suitable for plant operating conditions. The usual procedure is to heat the gelatin to a temperature of 60 to 71.1°C.(140 to 160°F.), then the carbon is added and the mixture agitated for from 30 minutes to one hour, and finally it is filtered.

Lard is also being successfully treated with activated carbon for removal of the typical lard odor which becomes so apparent when it is heated. After such treatment lard has an absolutely neutral flavor and is comparable to the hydrogenated shortenings produced from vegetable oils.

As we know, oils and fats frequently develop an undesirable rancidity. This rancidity is usually due to the oxidation of only a small part of the constituents of the oil or fat. These oxidized bodies can be removed with activated carbon, and the filtered oils will generally be of equal quality with the fresh oil. "Sweet pickle" lard, for example, can be made much more salable by treatment with activated carbon which brings its flavor up to a standard equal to that of the so-called "killing" lard.

There is the case of sorghum syrup, which in the untreated form has an unpalatable flavor for most people. By the use of carbon this flavor has been improved to such an extent as to greatly increase the market for sorghum.

Considerable quantities of activated carbon are used for the improvement of fruit juices, notably pineapple juice, to effect an improvement in flavor.

All these uses cover actual commercial operations, and many others might be named, but the above examples indicate the wide variety of products the flavor of which can be improved by the use of activated carbon.

OBTAINING A PANEL FOR JUDGING FLAVOR IN FOODS [1]

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(Received for publication, May 22, 1937)

Last year in a coöperative study among the Bureaus of Agricultural Economics, Chemistry and Soils, and Home Economics, King, Coleman, and LeClerc (1937) reported on the detection of relative effects of certain factors on bread flavor. Ninety-six individuals, fairly representative of one consumer group, were used as judges.

It was found that the group was not sufficiently discriminating to detect in the taste and odor of bread small differences which might have been caused by the factors studied, and that only a very small percentage could duplicate their judgments. A comparison of the results from the 96 judges with results from 16 of the group who were experienced in judging foods shows that while the expert judges corroborated the results of the whole group, they, too, were unable to detect small differences in taste and odor or to duplicate their judgments to any greater extent.

It was decided, therefore, before further bread-flavor studies were made to determine whether, by selection of a group with a high degree of acuity of taste and smell, more consistent judgments could be obtained.

LITERATURE ON CHEMISTRY OF TASTE

Many observations have been made on the tastes of substances. Blakeslee (1932), Fox (1931), Snyder (1931), and Levene and Anderson (1931) have studied especially phenylthiocarbamide and its derivatives. Blakeslee determined the threshold concentrations of aqueous solutions of phenylthiocarbamide at which a bitter taste was distinguishable by a group of children and their parents. Of a group of 283 individuals tested, 32.2 per cent were taste blind. From work on p-ethoxyphenylcarbamide Fox found 40 per cent of the individuals tested to be non-tasters. This work was verified by Snyder, who tested 440 individuals, finding 31.5 per cent to be non-tasters. Levene and Anderson, also using p-ethoxyphenylcarbamide, found in a study of 180 full-blooded American Indians 6 per cent to be taste blind as compared with 10.4 per cent in a group of 150 white individuals living in a nearby community.

[1] Read before a symposium on *Flavors in Foods* at the Chapel Hill, North Carolina, meeting of the American Chemical Society, April 12 to 15, 1937.

Some threshold values for the four recognized primary tastes, as reported by Parker (1932), Blakeslee (1931), and Crocker and Henderson (1932), are given (Table 1).

Mee (1934), reviewing the literature on chemistry of taste, states that it is a subject still in its infancy and that "when we have a rational standard upon which to base a scale of taste and the personal element can be eliminated we still have to discover the relationship between the arrangement of the groups in the molecule of the compound and its taste, before any comprehensive theory can be drawn up."

PROCEDURE FOLLOWED TO OBTAIN PANEL

A preliminary questionnaire was sent to the 96 judges participating in the earlier study on bread flavor for information concern-

TABLE 1

Thresholds of Taste Reported in the Literature

	Parker (several sources)	Blakeslee (21 individuals)	Crocker and Henderson (2 individuals)
	Moles per liter	*Moles per liter*	*Moles per liter*
Sodium chloride	.040	.0214 to .0854	.030
Sucrose	.020	.....	.020
Saccharine	.....	.00007 to .00055	.....
Hydrochloric acid	.0025	.0078 to .0622	.....
Tartaric acid	.....	.....	.00125
Quinine hydrochloride	.00004	.0004 to .006	.....
Caffeine	.....	.....	.0002

ing susceptibility to head colds, smoking habits, age, sex, quantity of bread habitually consumed, and prejudices against the flavor and odor of bread. Those persons having numerous head colds or any dislike for bread were checked off the list, leaving 64 individuals. This group was composed of 35 women and 29 men; 52 of the group were more than 30 years of age; and 26 smoked tobacco. More than half of the group were laboratory workers in various fields; the others were statisticians, stenographers, clerks, and executives.

This group was tested in duplicate for sensitivity of taste for chemically pure solutions of sodium chloride, sucrose, lactic acid, and caffeine. The minimum strength solution tested for each substance was .00005 M. Each solution in every series was twice the strength of the preceding one. The solutions of the substances being tested were put in numbered liter flasks and tasted in order of increasing strength.

The temperature of the solutions was approximately 25°C.(77°F.), since as Komuro (1921) reports, in the reactions of the sense of taste there is a temperature coefficient that functions below 20°C.(68°F.)

and above 30°C.(86°F.). The sensitivity increases from 10 to 20°C. (50 to 68°F.) and slightly diminishes between 30 and 50°C.(86 and 122°F.).

An attendant measured out five milliliters of the solution for each individual as he was ready for the test. The 64 judges were divided into groups of eight or ten for testing. Test periods were set two to three hours after meals: at 10 A.M., 11 A.M., 2 P.M., and 3 P.M. Each person was told that he was being tested for sensitivity of taste to the four known primary tastes—salt, sweet, sour, and bitter—but he was given no clue to the order in which the substances were offered. The instruction and record card used for the test is shown (Fig. 1).

BREAD FLAVOR COMMITTEE

Name of Judge.....Substance.....Date.....Time.....

Procedure: Rinse the mouth thoroughly with distilled water, discarding the water. Pour 5 c.c. of Solution No. 1 in a beaker, and then put it in your mouth. Swish the solution around so that it reaches the back part of the tongue. Discard. Record taste. Rinse the mouth with distilled water again. Wait two minutes then taste Solution No. 2. Use a clean beaker for each solution. Continue through the series with the same procedure.

Insert number designating the intensity of taste of the numbered solutions, using following key:

0—No taste
1—Very faint
2—Faint
3—Easily noticeable
4—Strong
5—Very strong

Solution No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Intensity																	

Desirability: Mark with a check (√) the most desirable intensity in the series for flavoring food.

What was the taste?.....

Number of solution at which taste was first identified.....

How long since smoking before taking the test?.....

How long since eating before taking the test?.....

FIG. 1. Instruction and record card for taste test of chemically pure substances.

The group was also tested for acuity of sense of smell, using the substances suggested by Elsberg, Brewer, and Levy (1935) for test-

ing smell sense by sniffing. These substances were benzaldehyde (almond-oil odor), citral (lemon-oil odor), coffee, menthol, oil of turpentine, and a 10-per cent aqueous solution of ammonia. In addition, a yeast dispersion in water and 95 per cent alcohol were included in the series, since these substances are odorous constituents of bread. The test materials were placed in test tubes, covered with cotton, and the tubes were stoppered. The lower part of the test tubes were wrapped so that the substances could not be identified by sight. Each individual recorded the odor of the substance in each test tube as judged by sniffing.

Twenty-six desirable judges were selected according to their ability to identify by taste the substances in solution and also according to their consistence, that is, their ability to duplicate judgments. One of these, a man, was eliminated for poor sense of smell. The remaining 25 included 14 women and 11 men, 12 of whom were smokers

TABLE 2

Quantities of Substances in Bread Rated According to Increasing Amounts

Substance	Sample No. of bread			
	1	2	3	4
	pct.	*pct.*	*pct.*	*pct.*
Sodium chloride	0.75[1]	1.5	2.25	3.0
Sucrose	0.0	3.0	6.0	9.0
Lactic acid	0.0	0.105	0.21	0.315
Caffeine	0.0	0.067	0.135	0.271

[1] All percentages based on flour.

and 13 non-smokers. Nineteen individuals of the group were more than 30 years of age. For sex and the use of tobacco the ratios were practically the same as in the original group of 64, but the proportion of individuals over 30 years of age was lowered.

The group of 25 was tested further to establish its discriminatory power by tasting, in duplicate, bread samples containing different quantities of sodium chloride, sucrose, lactic acid, and caffeine (Table 2).

Elimination of the less discriminating persons from the group of 25 was made on the basis of their ability to rate correctly the 32 slices of bread according to increasing quantities of the test materials added to it. No correlation was found between the group's consistence in judging solutions and its ability to rate correctly the samples of bread containing the various quantities of sodium chloride, sucrose, lactic acid, and caffeine. Fifteen scored above 65 per cent. One of these was removed from the panel because her sense of smell was considerably below the standard for the rest of the group. The final

group of 14 consisted of 11 women and 3 men, of whom there were an equal number of smokers and non-smokers, and 10 were more than 30 years of age.

In the earlier study one factor which was on the border line of significance was the effect of the method of mixing the bread dough on the desirability of odor and taste of the bread. Therefore, samples of bread mixed by the sponge method and by the straight-dough method were again judged in duplicate by the original 96 judges. The results of the ratings for taste and odor by the panel of 14 were analyzed separately for the purpose of comparing their consistence and power of discrimination with those of the original group of bread judges. Significant differences between the two differently mixed samples were not found by either group. A factor which had been found in the earlier study to have a significant effect on the odor and taste of bread was its freshness. Hence, the original 96 and the selected 14 were tested for their reactions to bread that was 12 hours old and that which was 24 hours old.

DISCUSSION OF RESULTS

In the records of the taste tests of the chemically pure solutions of sodium chloride, sucrose, lactic acid, and caffeine no cases of taste blindness for any of the four substances were found. All 64 individuals were able to identify the substances at some concentration submitted for tasting. However, there were cases of taste indiscrimination below the level of recognition, especially for salt and acid. Such cases have also been reported by Blakeslee (1932). Fifteen of the group recorded the taste of the salt solution in concentrations below the ones identified as salt, as "sweet" or "sour". A few persons recorded them as "bitter."

Nine called the acid solutions "bitter" up to the point of correct identification; three called them "sweet." Although Parker (1932) states that insipidity, such as is characteristic of distilled water, is probably real tastelessness, many in this study found the redistilled water used for rinsing the mouth to have a bitter taste. Calling the acid-test solution "bitter" up to the point where its true taste was recognized may conceivably have been due to taste response to the water in which the acid was dissolved. Crocker and Henderson (1932) found that it took several days for them to build up a reaction that enabled them to think of distilled water as tasteless.

Five individuals described as "bitter" the sweet solutions in concentrations below those accurately identified, and one individual thought the bitter solutions tasted "sweet" in the pre-identification concentrations. There was no way of knowing whether or not the

judges were trying so hard to taste something in the weaker solutions that their sensations of taste were psychological rather than real. Five individuals showing great confusion in taste reactions were eliminated before the records were analyzed. The data reported are for the remaining 59.

For purposes of interpretation the lowest concentration of any substance at which each individual identified it was considered his threshold of taste. No individual duplicated his threshold for all four substances. By subtracting the identification numbers of the solutions in the first and second test and the numbers of the solutions called "easily noticeable," and adding them together, the individu-

TABLE 3

Distribution of Numerical Consistence for 59 Individuals

Consistence (range of error)	"Easily noticeable"	Identification
1	3	3
2	8	8
3	18	7
4	12	8
5	7	11
6	2	8
7	5	5
8	3	5
9	1	3
10	0	0
11	0	1

al's consistence was determined numerically. On the whole the tasters were more consistent in their description of solutions as "easily noticeable" than they were in identification of the substance (Table 3).

In eliminating judges it was necessary to consider not only the size of the consistence figure but also whether it was distributed uniformly among the substances tasted. It was thought a fairly high consistence for all substances would be more desirable in a judge than a high consistence for three substances and a low consistence for the fourth.

To classify the individuals for sensitivity of taste to the different substances, the geometric mean of each individual's tests was taken as a representative value for that individual.[2] From the frequency distribution of these representative values, the position of the middle

[2] The writer is indebted to Charlotte Chatfield and Eleanor Hunt, Bureau of Home Economics, and Alexander Sturges, Bureau of Agricultural Economics, for statistical assistance.

50 per cent of the values was calculated (Figs. 2, 3, 4, 5). The values falling between the first and third quartile were termed medium, the ones falling above the third quartile were high, and those below the

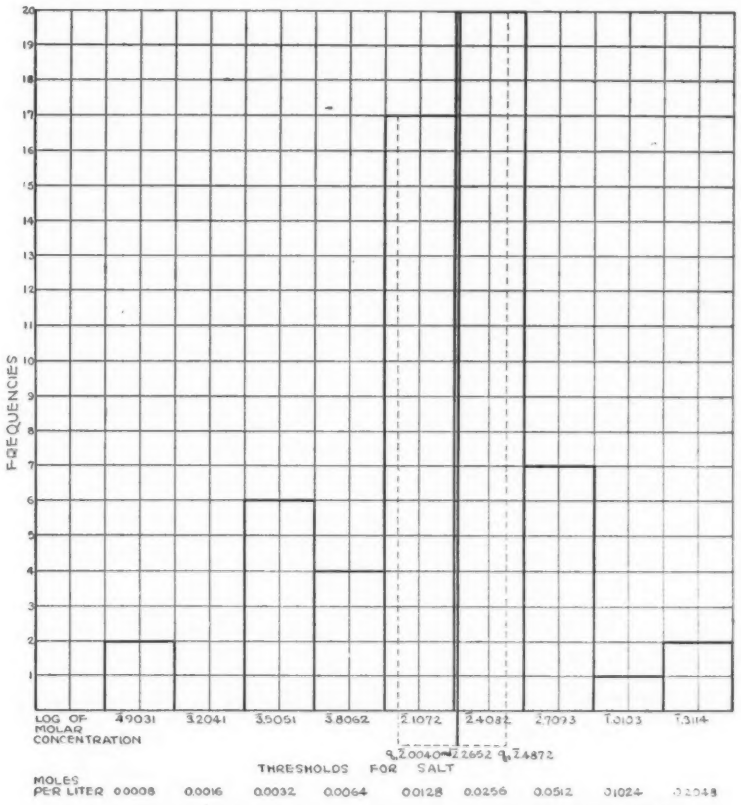

FIG. 2. Frequency distribution for the average threshold of 59 individuals.

first quartile were low. The distribution of thresholds of taste for the 59 individuals for each substance was as follows:

	Low	Medium	High
Sodum chloride	20	29	10
Sucrose	16	29	14
Lactic acid	17	29	13
Caffeine	15	29	15

The range of thresholds of taste in terms of the concentration of the solution tasted for each substance is shown (Table 4). In the

case of the sodium chloride the calculated mean fell at .0141 M, a solution which was not actually used in the test. Of the whole group, five had a low threshold of taste for all substances, five a medium threshold of taste, and two a high threshold of taste. The remaining

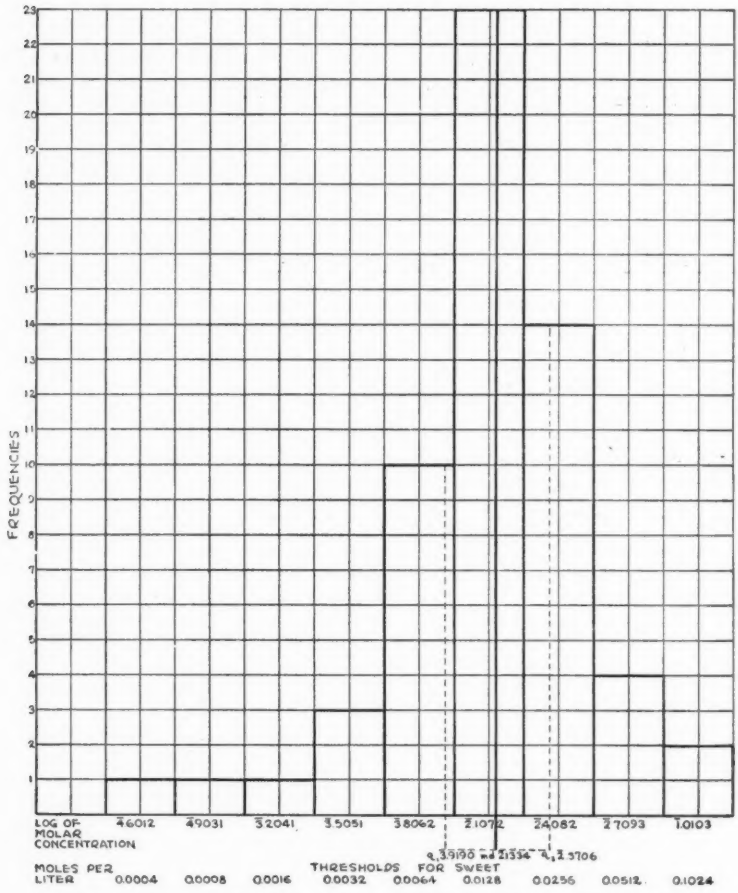

FIG. 3. Frequency distribution for the average threshold of 59 individuals.

47 had mixed thresholds for the substances, usually a combination of low and medium or medium and high.

The distribution of thresholds in the selected panel of 14 was as follows: two, low for all substances; two, medium; and one, high. The remaining nine had mixed thresholds.

Variance analysis was used to determine the effect of smoking, age, and sex on the individual threshold of taste of the group of 59. There were no significant differences in threshold for sweet, salt, or

TABLE 4

Concentrations in Moles per Liter of Solutions Ranging from Lowest to Highest Threshold of Taste

	Lowest	Medium	Highest
Sodium chloride	.0008 M	.0128 to .0256 M	.2048 M
Sucrose	.0004 M	.0128 M	.1024 M
Lactic acid	.0002 M	.0016 M	.0128 M
Caffeine	.0002 M	.0032 M	.0128 M

bitter; nor were there any differences in acid threshold so great that they may not have been due to errors of sampling (Table 5). The judges under 30 years of age in this group had a lower acid threshold

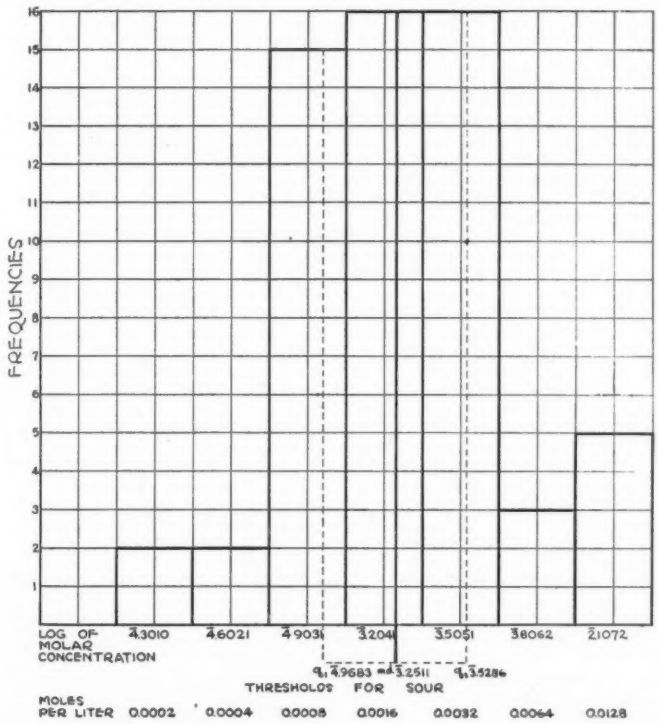

FIG. 4. Frequency distribution for the average threshold of 59 individuals.

than those over 30, and the difference was too great to be ascribed to experimental error. Experimental error as used here relates to the ability of these judges to duplicate themselves. Using the ordinary formula for testing the significance of differences between means, it can be shown that the ratio of the difference to its standard error

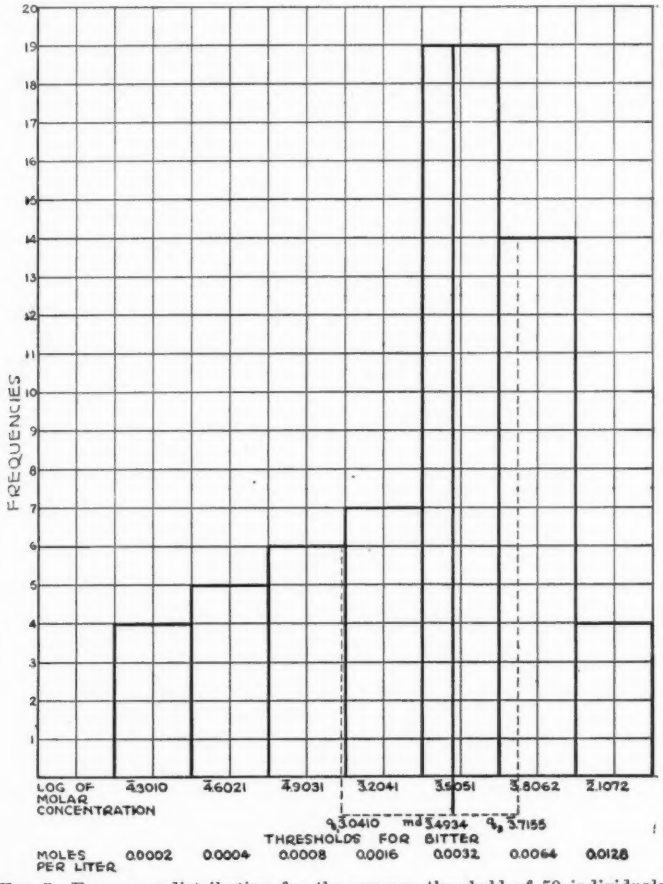

FIG. 5. Frequency distribution for the average threshold of 59 individuals.

is 1.35 when each judge's average of two tests is treated as an original observation. This indicates that such a difference may be expected to occur, owing to chance alone, more often than one time in ten.

Blakeslee and Salmon's (1931) results suggest that females are somewhat more acute tasters than males. Boelcke and Routh (1932),

in an investigation to discover the very weakest concentration of various common acids that could be detected by the human tongue, found that the group which could taste the weakest solutions were non-smokers.

TABLE 5

Analysis of Variance of Threshold of Taste for Sour

Source of variation	Degrees of freedom	Sum of squares	Mean square	F[1]	F[2]
Total	117	21.4571	.1834	.....	.....
Between means of smokers and non-smokers	1	.1231	.1231	1.98[3]	1.47[3]
Between means of males and females	1	.1794	.1794	2.88[3]	1.01
Between means of judges under and over 30 years	1	.5625	.5625	9.04[4]	3.11[3]
Experimental error (between duplicate determinations)	59	3.6698	.0622	.....	.....
Remainder	55	16.9223	.3077	.....	.....
Remainder plus experimental error	114	20.5921	.1806	.....	.....

[1] Using experimental error as the basis of measuring significance. [2] Using experimental error plus remainder as the basis of measuring significance. [3] Not significant. [4] Significant.

The data of the judgments of the whole group as compared with those of the selected panel on duplicate samples of bread made by the sponge and straight-dough methods and on 12-hour and 24-hour

TABLE 6

Difference Between Proportion of Judgments by the Whole Group and the Selected Panel on Duplicate Samples of Bread Made by Straight-Dough and Sponge Methods and on 12-Hour and 24-Hour-Old Bread

	Sponge *versus* straight-dough bread			12-hour *versus* 24-hour-old bread		
	Number of judges	Difference	Standard error	Number of judges	Difference	Standard error
Whole group						
Odor	74	0	....	77	2.84	±11.36
Taste	72	8.5	±11.7	72	3.24	±11.61
Selected group						
Odor	14	25	±25	12	37.1	±25.86
Taste	14	28.6	±22.9	13	9.56	±26.89

bread are shown (Table 6). The ordinary method for determining the significance of the differences was used. If the difference between the selection of the two loaves is less than some three times the stand-

ard error, the difference in choice between the odor and taste of the two loaves is not significant. It is evident that neither group of judges was able to make a significant choice in either experiment. The selected panel was somewhat better able to duplicate its judgments for taste of the bread than the larger group.

SUMMARY

In order to obtain a panel for judging flavors in bread 64 individuals were tested for the acuity of their sense of taste and smell. To test sensitivity of taste they evaluated their sensations and identified in duplicate, solutions of chemically pure sodium chloride, sucrose, lactic acid, and caffeine, tasting them in order of increasing strength.

No cases of taste blindness were found for any of the four substances, since all individuals were able to identify the substances at some concentration submitted for tasting. Many cases of taste indiscrimination were found, especially for salt and sour. The thresholds of taste for the four substances ranged from lowest to highest as follows: salt, .0008 M to .2048 M; sweet, .0004 M to .1024 M; sour, .0002 M to .0128 M; bitter, .0002 M to .0128 M. Of the whole group only five had a low threshold of taste for all substances, five a medium threshold of taste, and two a high threshold of taste.

Elimination of the least consistent and least discriminating judges in all the tests reduced the group of 64 to a final panel of 14 judges.

Comparison of the judgments of the whole group with those of the selected panel on duplicate samples of bread made by the sponge and straight-dough methods and on 12-hour and 24-hour bread showed no significant differences in their choice between the taste and odor of the breads in either test. The selected panel was somewhat better able to duplicate its judgments for taste of the bread than the larger group.

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FLAVOR OF ALCOHOLIC BEVERAGES[1]

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(Received for publication, May 22, 1937)

Flavor is considered a combination of odor and taste; aroma is that quality which affects the olfactory sense. The taste of a liquor is dependent also on such factors as degree of acidity, bitterness, and astringency, qualities not perceptible to the nose. Aroma or "bouquet" is due to volatile constituents, and in the bouquet of alcoholic beverages the various esters and higher alcohols are most important.

Many important factors enter into the development of a pleasing bouquet or aroma in alcoholic beverages. The naturally occurring volatile substances, particularly in fruits, play a part in influencing the aroma and flavor of the fermented beverages (wines and brandies).

It is the purpose of this paper to discuss the factors entering into production of flavor, and more particularly aroma or bouquet, in fermented beverages. These factors will be taken up in the following order:

1. The naturally occurring flavoring constituents, especially of fruits, which are influenced by variety, soil, climate, season, degree of maturity, etc.
2. The variety of yeast used in fermentation.
3. The effect of aging in wood.
4. Changes in flavor during storage in bottles.

COMPOSITION OF FLAVORING CONSTITUENTS

Information on the composition of the volatile flavoring constituents of fruits is quite meager. Probably some investigations have been made, the results of which have been withheld from publication for business reasons. The actual identification of the principal flavoring constituent of a fruit appears to have been first accomplished by Kleber (1913), when he identified amyl acetate as a constituent of the banana.

[1]Read before a symposium on *Flavors in Foods* at the Chapel Hill, North Carolina, meeting of the American Chemical Society, April 12 to 15, 1937.

The investigations of Power and Chesnut (1920, 1921a, 1922) have shown that apple oil contains amyl formate, acetate and caproate, acetaldehyde and geraniol; and that the volatile oil from peaches contains the linalyl esters of formic, acetic, valeric, and caprylic acids with a considerable proportion of acetaldehyde. The same authors (1921b) have separated methyl anthranilate from some varieties of grape, particularly the Concord.

In a study of the distribution of volatile flavor in grapes and grape juices, Sale and Wilson (1926) have determined methyl anthranilate, volatile esters, and volatile acids in 84 samples, representing about 55 varieties of grapes. Their data indicate that *vinifera* grapes do not contain methyl anthranilate and that the largest amount is in Concord, Stray, and Niagara. Campbell grapes, while containing no methyl anthranilate, were found to be much higher than any of the others in volatile ester content, the amount of which was found to vary widely. They found that nearly as much volatile flavor remains in the pomace as is contained in the juice, and that, contrary to general opinion, the volatile flavor of grapes is not contained almost wholly in the skins.

Morgan (1934), in a general article, states that pineapple oil contains ethyl butyrate, amyl acetate, amyl butyrate, and acetaldehyde; that pear oil contains amyl acetate; and that strawberry, currant, cherry, apricot, plum, and gooseberry oils have all been prepared and certain constituent chemicals identified in each case. Unfortunately no references are given in this article.

Aside from the work on methyl anthranilate, no information was found on the other constituents of the flavor of grapes, and little on the constituents of the volatile flavor of wines.

In a study of an old Sicilian wine (Castel Vetrano), Scurti (1910) found that the volatile esters consisted principally of ethyl acetate with some ethyl isobutyrate and lesser amounts of caprylic and capric esters. Butyric acid and nonyl esters were not present. Small amounts of aliphatic aldehydes and furfural were identified but he found no oxyaldehydes.

Bottini (1924), studying the esterification processes occurring during the aging of Barbera wines, recognized ethyl acetate, ethyl isobutyrate, amyl alcohol, ethyl succinate, and small amounts of the esters of higher fatty acids in the flavor isolated from a sample of old wine. He found volatile esters almost entirely absent in the young wines and those in the old wines increasing in direct proportion to the volatile acidity and inversely to the cream of tartar.

VARIETIES OF YEAST IN FERMENTATION

When fruit juices are fermented in the production of wines and brandies, the natural flavors are carried over into the finished product, influencing the character of its flavor and aroma. The natural flavoring constituents of some fruits, however, are not always desirable in an alcoholic beverage; for example, the "foxy" flavor of wines made from some varieties of grapes. During fermentation the yeasts exert an action in the development of bouquet, and the variety of yeast used has a profound influence on this quality of the finished product. Kayser (1924) has given a very good summary of the results of investigations on influence of yeasts on bouquet of wines. Numerous experiments have shown that different selected yeasts do not give the same products under equal conditions on the same must. They are not equally able to decompose the nutritive materials present. On the other hand, the same yeast may act very differently, not only on different musts but on the same must when fermentation conditions are varied.

Yeasts form, in variable proportions, volatile products which enter into the bouquet of fermented beverages and affect their quality. The proportions of these products depend on the variety of yeast, composition of the must, and conditions of fermentation (temperature, duration, aeration, etc.).

The formation of volatile acids is influenced by the variety of yeast. Thus the elliptical yeasts have been found to be less productive of volatile acids than the apiculate forms under the same conditions. Volatile acids do not come exclusively from sugar; many yeasts are apt to decompose fixed acids or even to oxidize alcohol and acetaldehyde.

Yeasts also influence the production of aldehyde, the proportion varying with the variety of yeast and the conditions under which it acts, according to Roeser (1893) and Trillat (1908). Aldehydes form acetals, at least in part, in wines and spirits, and the acetals resulting contribute to the formation of bouquet.

Pure yeasts also form higher alcohols, the amount varying with the variety of yeast and the composition of the must. Ehrlich (1906, 1907) has shown that the higher alcohols are formed at the expense of the amino acids (leucine, isoleucine, valine), producing isoamyl, active amyl, and isobutyl alcohols.

Esters, probably the most important factors in producing the proper bouquet of fermented beverages, are formed by chemical or biological processes. Their production by fermentation depends on the variety of yeast used and the nitrogenous foods available.

The addition of ammonium sulfate and ammonium phosphate facilitates esterification, sometimes to a considerable extent. Thus, according to Kayser (1924), an addition of .5 per cent of nitrogen in the form of ammonium sulfate has caused an increase of volatile esters from 57 to 162 milligrams and in the form of ammonium phosphate from 93 to 333 milligrams; and yeasts from fine grapes are more apt to develop esters if the composition of the must is in harmony with their requirements. The selected yeasts, to generate bouquet, must have sufficient amino acids, producing higher alcohols, which, entering into combination with the diverse volatile acids, produce esters.

With regard to the character of the bouquet formed by the use of selected yeasts there are differences of opinion, some holding that the particular yeast used will engender a bouquet similar to wine from the grape from which it originated, though used in fermenting the must from a mediocre grape. Yet it seems to be agreed that the use of a properly selected yeast results in a better wine with more bouquet.

Rosenstiehl (1902, 1908) takes the rather extreme view that the difference between wine made from fine grapes and ordinary grapes is due to the yeast growing on the grapes more than to the quality of the fruit itself. It is self-evident that bouquet principles owing to the action of the yeast itself are less evident when the variety of grape is rich in natural aroma, as this will mask aroma developed by the yeast. This is noticeable with very aromatic varieties of apples.

Kayser (1924) states that experiments have shown that apple and pear juices, seeded with wine yeasts, have a vinous taste and aroma recalling the origin of the yeast and that the yeasts of fine vineyards can generate a very pronounced amount of volatile esters in the must of very mediocre grapes.

As regards the importance of esters, opinions are divided. Schmitt (1892), having made complete analysis of wines from the ducal cellars of Nassau, concluded that there exists a direct relation between the bouquet of wines and the amount of volatile esters. Mach (1893) has agreed that this interpretation is justified in a certain measure, though there are often exceptions; while Gayon (1903) has found for the wines of Bordelais that those presenting the better bouquet are not always the richest in esters. He has found a certain parallelism, especially in old wines, between the esters and the sum of alcohol plus total acidity. In fact the *nature* of the volatile esters is more important than their *quantity*, considering effect on bouquet.

Cordier (1899) believes that there exists in grape must a substance, or group of substances, resistant to a heat of 46.1°C.(115°F.)

and apt to give rise to odoriferous substances under the influence of yeast. He adds that certain yeasts are more apt to develop this bouquet; they have decided aroma-producing ability.

Müller-Thurgau (1893-1894) believes that the bouquet question is not settled and that the exaggerated, widespread ideas, chiefly emanating from France and which he opposed, are already much discredited. He says that today no expert expects to get a wine with Riesling bouquet from any white grapes by use of Rhine wine yeast, or from blue grapes, of whatever origin, to produce a fine Bordeaux wine with Bordeaux yeast. He does agree, however, that odor and taste of wine fermented with pure yeast are better than the same wine made without pure yeast and that odor and taste are influenced variously according to the species of yeast.

Jacquemin (1899) made experiments indicating that non-odorous compounds, such as glucosides, break down under the action of a diastase secreted by the yeast, forming sugars and aromatic substances. This brings us to another phase of the matter, the action of enzymes on wine, of which we know little, specifically, at present.

EFFECT OF AGING IN WOOD

During aging in wood, discussed by Crampton and Tolman (1908), some flavoring materials enter into the liquor from the cask; but the main effect is a slow oxidation owing to osmosis through the wood. This leads to a mellowing of the product and the formation of some aldehyde. The true, full bouquet of wines, however, develops on storage in bottles, and in bottles the aroma is confined and cannot be lost by evaporation.

EFFECT OF STORAGE IN BOTTLES

Rosenstiehl (1902, 1908) observed that the true bouquet developed only after several months and that it was preceded by a rather disagreeable bouquet and musk-like taste. Each time this experimenter failed to observe this taste he failed to develop the bouquet later.

The observation of the musk-like taste preceding the true bouquet seems to confirm Ehrlich's view, which attributes justly to certain yeasts the faculty of effecting the first transformation of nitrogenous substances necessary to development of bouquet. In the development of bouquet in bottles, enzymes undoubtedly play a part.

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CHEMICAL DETERMINATION OF AROMA IN BUTTER AND BUTTER CULTURES[1]

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(Received for publication, May 22, 1937)

INTRODUCTION

It has been aptly said that "flavor is the voice of food." The analogy seems a proper one, for like the voice, flavor can be repulsive, pleasing, or indifferent. It might be more exact, perhaps, to compare flavor with a chorus of voices capable either of harmonious blending or of irritating discord, not to mention the various degrees within these two extremes. Furthermore, when we consider the varying and variable tastes and preferences of the audience, we eventually arrive at the conclusion that a veritable myriad of psychological and physiological factors are involved.

All this points to empirical procedures rather than exact chemical methods, for in judging the quality of a food product the average consumer unquestionably is influenced by the attractiveness of its price and the neatness and appeal of its appearance as well as the character of its flavor or taste. Variety may be the spice of life, but with a staple food like butter, experience has demonstrated that uniformity is probably even more important than the excellence or delicacy of its flavor. Such appears to be the criterion of the great majority of the consuming public.

In trade practices ample confirmation is found of this common trade experience. Butter, for example, has been graded for years by the skilled taste sense of commercial and official judges on the arbitrary and numerical basis of 45 points for flavor, 25 for body, 15 for salt, 10 for color, and 5 for package. Analyze these terms broadly and you will find that uniformity is their virtue, at least for the last three named. Proceed even further, cross-examine particularly the commercial judges, and invariably you will find that the source of production of the butter is considered as well as a prediction of its keep-

[1] Read before a symposium on *Flavors in Foods* at the Chapel Hill, North Carolina, meeting of the American Chemical Society, April 12 to 15, 1937.

ing quality—uniformity again. Going from one market to another will reveal the truth of the adage, "one man's food is another man's poison," for butter scored 90 points in some sections will be accorded 93 points in another region. Yet in spite of these differences, there is remarkable agreement of scores among skilled graders in a given market. The reason for this appears to be that certain flavors and aromas given the premium in some markets are discounted elsewhere.

Therefore, in its present stage of development the chemical determination of aroma in butter does not threaten to abolish the usefulness of the experienced butter judge. Aroma, after all, is the indigenous supplement of taste, for we must smell in order to taste, according to physiologists. If we can determine quantitatively a characteristic aroma, we are on our way in the chemistry of flavor analysis. As pioneers we are opening up new territory even though the vista may amaze and humble us in its extent of complexities. Yet we are probably facing no more prodigious task than confronted the first alchemists. Our generation will not reap the full reward but chemical exploration of the flavor development in butter will reveal new possibilities for the manufacturer which will enable him better to control its delicacy as well as its uniformity. In that connection the historical development of our present-day concepts of the flavor-producing factors in buttermaking will doubtless prove interesting as well as enlightening.

BEGINNING OF RIPENING PROCESS

As early as 1850, buttermakers learned that the flavor of butter could be definitely influenced by the acid fermentation, or souring, of the cream from which it was churned. This process of souring is generally called "ripening" by the buttermaker. The cream was allowed to sour or ripen naturally in order to improve the flavor of the butter. In this natural souring of cream lactic acid is formed as well as a variety of other products which are present either in small amounts or as traces. While the principal product is lactic acid, there is also present carbon dioxide, hydrogen, acetic acid with traces of propionic and possibly butyric acids, as well as the products of protein cleavage including amino acids as well as more complex compounds. Obviously such a variety of products, particularly those capable of producing bitter or otherwise disagreeable flavors and odors, led to the use of soured milk in the hope of better control as well as the desire to hasten the process. This souring agent, however, did not produce uniform results. In 1884, Storch, a Danish bacteriologist, began a bacteriological study of butter and demonstrated that presumably pure cultures of lactic-acid-forming bacteria could be used as an effective souring agent for pasteurized cream. We say "pre-

sumably pure cultures of lactic-acid-forming bacteria" advisedly, because Storch was not aware at that time that the flavor of butter cultures (i.e. the souring agents for the cream to be churned into butter) depended upon the symbiotic activities of two or more types of bacteria.

A vast number of scientists have made valuable contributions to this subject but the discovery in 1919 by Hammer and Bailey in the United States and by Boekhout and deVries (1919) in Holland proved that the desirable flavors in butter cultures depended upon at least two different types of bacteria. In Holland ten years later van Niel, Kluyver and Derx (1929) were studying propionic acid fermentation processes and discovered that diacetyl was responsible for the characteristic aroma of fine butter. They found from two to four parts per hundred thousand of diacetyl in fine butter and when these concentrations were added to a butter neutral in flavor, there was developed a flavor and odor unmistakably similar to that of good butter. They concluded that diacetyl is either responsible for the aroma of butter or is the principal component of the aromatic ingredients. They also later found that the bacteria causing fermentation of the citrates in milk were responsible for the production of diacetyl and associated neutral compounds, as shall be pointed out presently.

Diacetyl is considered to come from acetylmethylcarbinol because of the relatively rapid oxidation of the odorless acetylmethylcarbinol to the pungent, penetrating, odoriferous diacetyl. Michaelian, Farmer, and Hammer (1933) found that butter and butter cultures with satisfactory flavor and aroma contained considerable quantities of acetylmethylcarbinol plus diacetyl, while those lacking in flavor and aroma contained small amounts or none. The typical lactic-acid-forming bacteria, such as *Saccharomyces lactis* and *Saccharomyces cremoris* (Jensen), produce lactic acid with little if any flavor; while the citrate-fermenting bacteria, such as *Saccharomyces citrovorous* and *Saccharomyces paracitrovorous* (Hammer), are aroma producers but only produce flavor and aroma either when grown in symbiotic relations with the lactic-acid-forming bacteria or when milk is first artificially acidified to the proper hydrogen ion concentration.

MODERN CONCEPTION OF FLAVOR PRODUCTION

The modern conception of the mechanism of flavor production in butter cultures is about as follows: The *Saccharomyces lactis* grows rapidly at first, serving to discourage the development of contaminators, but primarily to produce lactic acid. This production of lactic acid lowers the pH to approximately 4.3 when the citrates normally present in milk become available to the citrate-fermenting bacteria in

the butter culture, or "starter," as the buttermaker calls it. Apparently these flavor-producing bacteria do not begin to function until an optimum acidity for their activity has been developed by the lactic-acid-forming bacteria, both of which types are present in a satisfactory butter culture. As soon as the fermentation of the citrates begins, appreciable increases in the amount of acetylmethylcarbinol are produced. Concurrently there is a development of volatile acids, principally acetic and some propionic. Acetylmethylcarbinol appears to be the first neutral compound produced by the citrate-fermenting bacteria; there is then presented the possibility of oxidizing some of the carbinol to diacetyl or of reducing it to 2,3-butylene glycol. An oxidation is evidently the preferred change since it yields diacetyl, which is the compound having the desired aroma; while the reduction yields the glycol, which is probably never oxidized to diacetyl in a butter culture under practical conditions.

To reiterate, the definite chemical compounds formed during the ripening or souring of a butter culture, according to Hammer (1936), are as follows:

Non-volatile acid
  Lactic
Volatile acid
  Acetic
  Propionic
Gas
  Carbon dioxide
Neutral compounds
  Diacetyl
  Acetylmethylcarbinol
  2,3-butylene glycol
Protein decomposition products
  Amino acids and also more complex compounds.

Probably this list does not include all of the chemical compounds produced in a butter culture, and additional research may extend the number.

So far we have considered the chemical changes which take place during the ripening of a satisfactory butter culture produced from milk, as mentioned earlier. It should be stated at this point that the milk is first pasteurized so as to destroy its normal bacterial flora before it is inoculated with a culture of bacteria which have been described as essential for a good butter culture or starter.

When the ripened butter culture, rich in acetylmethylcarbinol, diacetyl, acetic and lactic acids, is mixed with the cream to be churned,

there is distributed throughout its mass a portion of these substances. If the cream itself is then ripened, or allowed to sour (after its preliminary pasteurization so as to render innocuous its original bacterial flora), an increased production of the culture flavor constituents will obviously result. It is not possible to ripen cream, however, to the extent that is the practice with a butter culture because of the detrimental effects such fermentation would have upon the keeping quality of the resulting butter. Generally, therefore, cream well mixed with a good starter, or cream that has undergone limited ripening after its inoculation with a good starter, will churn into a butter containing appreciable amounts of the desirable flavor originally produced in the butter culture.

Michaelian and Hammer (1935) have reported that butter contains much smaller amounts of acetylmethylcarbinol and diacetyl than the cream from which it was churned. Butter has been found to contain from one-third to one-thirtieth of the amounts of acetylmethylcarbinol and diacetyl present in the cream. Most of the flavor is lost in the buttermilk, it having been demonstrated that from two to three times as much diacetyl and acetylmethylcarbinol is found in the buttermilk of commercial churnings than was present in the cream itself.

DISCUSSION OF CHEMICAL PROCEDURE

From the foregoing remarks it is evident that butter cultures and related dairy products inevitably contain more acetylmethylcarbinol than diacetyl, the former being the precursor of the latter. Schmalfuss and Barthmeyer (1932) have studied the presence of both acetylmethylcarbinol and diacetyl in various food materials and noted that those examined contained much more carbinol than diacetyl; in sour milk about ten times as much acetylmethylcarbinol as diacetyl was found. Hammer, Stahly, Werkman, and Michaelian (1935) investigated the phenomenon, observed after a maximum quantity of acetylmethylcarbinol and diacetyl had been produced in the ripening of a butter culture, of a decrease in the concentrations of these ingredients and reported that citrate-fermenting microörganisms are capable of reducing such neutral compounds to 2,3-butylene glycol and that such reduction accounts for the disappearance of acetylmethylcarbinol and diacetyl in butter cultures. Diacetyl being the important ingredient from the standpoint of flavor has led to the analytical procedure in which the most important reaction is the formation of the nickel salt of dimethylglyoxime which is formed in the presence of hydroxylamine hydrochloride and nickel chloride.

Diacetyl is an oily yellow liquid with a slightly greenish tinge. The color is due to the carbonyl groups in the alpha position, a

chromophoric grouping. Solutions of diacetyl diluted even to one part in one thousand parts of distilled water presents a beautiful greenish yellow florescence. Its vapor is the color of chlorine vapor and in concentrated form possesses a pronounced disagreeable odor but in a very dilute volume suggests the odor of fine butter. It is stable up to 80°C.(176°F.) and particularly so in an acid reaction. It is a little lighter than water, having a density at 22°C.(71.6°F.) of .97 and is readily soluble at ordinary temperatures in water.

The acetylmethylcarbinol and diacetyl are commonly determined together because diacetyl is ordinarily present in a dairy product in much smaller amounts than acetylmethylcarbinol. The reagents used are ferric chloride solution, 40 gm. to 100 ml. with distilled water; hydroxylamine hydrochloride solution, 20 gm. to 100 ml. with distilled water; sodium acetate solution, 20 gm. to 100 ml. with distilled water; and nickel chloride solution, 10 gm. to 100 ml. with distilled water.

Ordinarily a 200-gram portion of the material to be examined (cream, buttermilk, butter culture, etc.) is distilled with steam, after adding 40 ml. ferric chloride solution to oxidize the acetylmethylcarbinol to diacetyl. The distillate is collected in a mixture of hydroxylamine and hydrochloride and sodium acetate solutions by means of an adapter on the end of the condenser and the nickel chloride solution is added later. The quantities of reagents used depend on the amount of diacetyl expected. Generally in analyzing 400 gm. of butter or 200 gm. of butter culture, 4 ml. each of hydroxylamine hydrochloride and sodium acetate solutions plus 2 ml. of the nickel chloride solution will suffice. In the case of butter a 400-gram portion rather than a 200-gram portion is recommended because of the relatively smaller amount of acetylmethylcarbinol and diacetyl. One hundred and fifty milliliters of distillate is adequate and the distillate with the added reagents is allowed to stand undisturbed for 16 to 18 hours (over night) in order to permit complete crystallization. The nickel salt, brick-red in color, is then filtered into a weighed sintered glass Gooch crucible and washed thoroughly with distilled water, after which it is dried to constant weight at 105 to 110°C.(221 to 230°F.) and the results recorded as milligrams of nickel salt per 200 grams of material. The weight of nickel multiplied by .5958 will give the equivalent weight of diacetyl.

In the proper handling of butter cultures it is highly important to know that the microörganisms are actually functioning in active production of acetylmethylcarbinol and diacetyl. Starters which have become weak in flavor almost invariably are found to have degenerated into simply a culture of *Saccharomyces lactis* in which the *Sac-*

charomyces citrovorous and *Saccharomyces paracitrovorous* have become diminished in numbers to the point where their citrate-fermenting properties have become seriously impaired. Although the loss of flavor can be readily detected by one skilled in the art, it is advisable frequently to keep more accurate check on butter cultures by an accurate chemical analysis. Many butter manufacturers follow the practice of analyzing their butter cultures periodically.

On the other hand, the procedure of chemical analysis, as outlined, is somewhat involved and requires rather complete laboratory facilities. A simpler practical test has been devised by Hammer (1935) which consists of mixing equal quantities of butter culture and 40 per cent NaOH (about 2½ ml.), then adding a small piece of creatine about the size of a grain of wheat. Good starters which produce ample amounts of acetylmethylcarbinol and diacetyl develop in a few minutes a pink band at the surface, whereas lesser amounts of these compounds are indicated by a very faint pink band developing more slowly or perhaps no pink band at all, indicating the absence of such compounds. This test generally has qualitative value.

More recent observations by Blaisse and Martin (1936) have brought out a new carrier of the true butter aroma, namely methylglyoxal. Its name does not suggest any relation to diacetyl, yet the two substances are close chemical cousins. They can both be produced by the bacteria of milk. It is probable that further investigation will tend to indicate that this chemical will share with diacetyl some importance as a source of aroma of butter.

CONTROLLING UNIFORMITY OF BUTTER FLAVOR

An introductory statement referred to "new possibilities for the manufacturer to control not only the delicacy but the uniformity of butter flavor." So far in our discussion it is evident that this control applies only to attesting the viability of butter cultures from the standpoint of their flavor production. Obviously, the butter itself could not be standardized in flavor by such remote control of butter cultures. On the other hand, the use of synthetic flavors is not to be advocated because of the ruling by the chief of the United States Food and Drug Administration defining them as illegal. Furthermore, the use of diacetyl alone would not be favorably received by the industry because it imparts an artificiality of flavor which would not encourage the increased consumption of butter so prepared. Butter flavored with the aid of butter cultures possesses a flavor character lacking in a butter flavored only with diacetyl or similar synthetic materials.

This fact led to the very practical suggestion of Ruehe and Ramsey (1935) to utilize starter distillate for imparting to butter a fine flavor. These investigators found that a well-ripened butter culture, rich in its acetylmethylcarbinol and diacetyl as well as its volatile acidity constituents, could be steam distilled so as to recover these essential flavor ingredients in the condensed distillate. Furthermore, it was found that the direct addition of such distillate to the butter itself produced a result which is indistinguishable from butter produced from the same cream previously ripened with the butter culture itself. Obviously, such a method of flavoring butter has many advantages not possessed by the practice of using butter cultures for ripening the cream before churning. In the first place, there is no certain means for controlling the production of flavor in a butter culture *per se*, and consequently its standardization in the resulting butter is impossible. On the other hand, the butter culture distillate can be analyzed and standardized in its flavor constituents with the result that its incorporation in butter can be definitely controlled. The possibility of contaminating microörganisms influencing the quality of butter is eliminated in the use of butter culture distillate, a certainty which does not always exist when butter cultures are used for ripening cream to be churned into butter. This discovery by Ruehe and Ramsey (1935) promises to be an important development in the art of buttermaking because it permits the control of flavor in butter by means of accurate chemical analysis rather than by empirical methods—an important milestone in the evolution of the industry.

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SOME FUNDAMENTAL ASSUMPTIONS PERTAINING TO THE JUDGMENT OF FOOD FLAVORS[1]

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(Received for publication, May 22, 1937)

INTRODUCTION

A great deal of good chemistry has been very properly devoted to making the human foods better. Foods may be better in many ways: more abundant, more nutritious, cleaner, have greater keeping qualities, and finally have better flavor, in the broadest sense of this term. In regard to all of these points, except the last, there is little misunderstanding. Most scientists would agree as to how to go about studying questions of yield, nutritive quality, freedom from harmful bacteria, etc., and there would be general agreement on the significance of the results obtained. In the matter of better food flavors and better eating qualities, however, one finds a lack of clear understanding and one expects less agreement as to the conclusions to be drawn from data obtained.

It must be admitted that the determination of human preferences for food products is only partially a chemical problem. However, now that chemical laboratories have been invaded by rat colonies, X-ray apparatus, etc., we no longer have to apologize for the liberal inclusion of other sciences. As a matter of fact, a fundamental study of human preferences for foods draws liberally on many sciences besides chemistry, principally the following: physiology, to study the workings of nerves and organs; psychology, to explain the reactions to sensations supplied by the nerves; physics, to study the physical properties of foods; sociology, to cover the social reasons for food preferences; and finally mathematics, called into play for a statistical analysis of the data.

The literature on this subject is in fact scattered through the journals of all these sciences. This is one reason for the general lack of familiarity with the scientific foundations of this subject.

DEFINITIONS OF TERMS USED

Since there is some confusion in the use of terms pertaining to the "eating quality" of foods, the meanings used in this paper are defined as follows: "Taste" means taste proper, that is the effect of the food on taste buds of the tongue. "Odor" or "smell" means the

[1] Read before a symposium on *Flavors in Foods* at the Chapel Hill, North Carolina, meeting of the American Chemical Society, April 12 to 15, 1937.

effect on the olfactory organs of the nose. "Flavor" is used to represent the combined effect of the food on the taste and olfactory nerves. "Mouth feel" refers to the physical effect of the food in the mouth—tenderness, fineness, smoothness, and similar qualities. "Eating quality" includes the combined effect of taste, odor, and mouth feel. "Palatability," as suggested by Ingels, Irwin, and Landis (1936), is used as a synonym for eating quality. These meanings are somewhat confused by the fact that the terms are often used popularly in a less exact sense. Taste is sometimes used, for example, to mean taste proper; sometimes taste and odor; and sometimes the combined effect of taste, odor, and mouth feel.

Every student of the subject now realizes the great importance of odor in the palatability of food. Few recognize the importance or complexity of mouth feel. This includes many sensations brought to us by the tactile organs of the mouth and tongue and the muscular effort necessary for manipulating the food in the mouth. Some examples are fineness or coarseness, as in milk chocolate; toughness, as in meat; crispness, as in crackers; softness, as in cake; plasticity, as in cheese or ice cream; viscosity, as in cream; ease of disintegration in many foods; and amount of saliva necessary for swallowing, which Whymper (1919) has suggested as a measure for the staleness of bread.

The *amount* of some factors contributing to the palatability of foods may be measured objectively, such as acidity or saltiness affecting taste and volatile materials affecting odor. Physical properties of biscuits, bread, cake, meat, vegetables, etc., have been measured by Davis (1921), Platt (1931), and Platt and Kratz (1933). More rapid progress would be made if more chemical and physical measurements were made as a check against the organoleptic determinations when foods are judged.

The amount of some of these factors can be measured with scientific apparatus, but the *desirability* of these factors, Platt (1937), can be determined only by subjective means. Many factors cannot now be satisfactorily evaluated by any means except by the subjective, organoleptic tests. The measuring instrument is the human being, and human beings vary widely in sensitivity, that is, in the strength of sensations which they can distinguish. Even with exactly the same degree of sensitivity they vary in their preference—some like it sweet and some like it sour. Also, the same individual varies both in sensitivity and preferences according to his physiological condition. Finally there is a marked confusion between the sensation which a person really detects and the sensation which his imagination

suggests. It is out of this confusion of confusions that we are asked to bring system and scientific precision.

A fair analogy is as follows: Suppose we were asked to determine the relative weights of several nearly equal masses. Suppose we had before us 1,000 balances of all degrees of sensitivity, and the gram weights used were of all degrees of accuracy. The condition of any balance or weight might vary from time to time without notice. Suppose further that we had no Bureau of Standard Weights or any other standard of known accuracy to check against. That is the kind of situation to be faced when attempting to determine human preferences for foods.

CONSUMER PREFERENCE TESTS

Attempts to determine food preferences are frequently made. Some progress has certainly been achieved, as is shown by various papers on the subject which have been published. In tests where two or more foods are submitted for comparison, there are always more votes for one of the samples than for the others. One can, if he wishes, draw conclusions from such a vote, but the results are often not very convincing. If the test is repeated, one often finds a large difference between duplicates, and the more critically he examines the method used the less sound it seems. In fact some of the so-called consumer preference tests are perfect examples of "the search in a dark room for a black cat which isn't there."

This feeling is summed up by Ingels, Irwin, and Landis (1936) in the Report of the Bread Judging Committee (of the New York Section of the American Association of Cereal Chemists) which states: "A careful study of these twelve contests indicates that we have not made any decided progress in our ability to judge odor and taste in bread."

After going over a number of reports of such tests it seemed that progress could be made if one could set forth clearly exactly what he is trying to do and what assumptions are involved in the methods used. This should help to realize the possible error resulting from each assumption. A study of each of these errors would doubtless result in the discovery of means for reducing it. At least it should clarify ideas. This is in line with the work of Bell (1937) who said: "Only a decently critical familiarity with *all* the assumptions underlying a particular mathematical formula can teach us what not to take too seriously when the formula presents us with an impressive-looking number. Mathematicians are not, as a rule, credulous; their clients almost invariably are."

Much of the confusion which has existed in this field has come not from insuperable difficulties in technique but from a lack of clear

thinking in regard to just what is being attempted. In this field more than any other "a question clearly stated is half answered." Therefore, before taking up the fundamental assumptions let us examine the situation more clearly.

First we define the *"best" flavor or texture or eating qualities as the flavor, texture, or eating qualities which the public in question prefers in the given food.* In the end we have to fall back upon public preference as the ultimate authority. Except for this, there is no right and wrong in food preferences. Note, however, that the public does not necessarily represent all the people in the United States. We can and should define the public as clearly as possible in each problem. The more we can narrow down the public under consideration in a given test the simpler the problem becomes. A baker selling bread in Boston has a different public and a different "best" from a baker selling in Denver. The public to be considered for champagne should not include the poorer sections of society.

METHODS FOR JUDGMENT OF FOOD FLAVORS

There are two quite sharply defined methods for the judgment of food flavors and in general for the evaluation of the relative palatability of food products. In the first the standard of perfection has been agreed upon. The method consists in deciding how near the given samples of food come to the set standard of perfection. The most widely used example of this is the scoring of butter. Judges are schooled in points for scoring the ideal butter and in just how much to deduct for each fault in the samples scored. A somewhat illogical, but practical, system for the judgment of butter has been built up on the basis of which millions of pounds are sold.

Other products for which ideal standards are more or less widely accepted include milk, American cheese, ice cream, and bread. In such cases there is generally a limited number of specially instructed judges whose duty it is to determine how nearly a given sample comes to a predetermined standard. These judges have often had years of experience in such work. Where judgment can be left to a limited number of people who can be carefully instructed, a similar system of scoring can be adopted even when no ideal standard has been set. General directions for scoring food products have been described by Platt (1931).

The second general method is to appeal directly to the general public to see how well they like a given food or foods. Where the first method consults a few specially trained or specially instructed judges, the second method consults a very large number of the average public who are by no means experts. In fact in this case an average cross

section of the opinion of the public concerned is desired and not opinions of a group of self-styled experts. Obviously the instructions for judging to be given to a large number of untrained people must be few and very simple. An improved technique for carrying out tests by this second method has been described by Platt (1937).

This second method may be called a consumer preference test as contrasted with the method of scoring by a few specially selected judges. In the United States the final legal authority under the Constitution rests with the people. In the same way the final authority as to what is best in the palatability of foods rests with the opinion of the ultimate consumer. In the last analysis this opinion can be obtained only by some form of consumer preference test, formal or informal.

Often enough this test consists of offering the food product for sale. If it sells well, the conclusion is that the public likes it and vice versa. This is a most expensive and inefficient way to conduct such a test. It is inefficient because it does not necessarily answer the question as to whether or not the public likes the food. Low sales may be due to high prices, poor distribution, unattractive package, or many other causes entirely separate from palatability.

Where food is scored according to a fixed standard by trained judges, as in the case of butter, it is tacitly assumed that the standard fixed is the one which most people prefer. There is no meaning to a standard with any other basis. If one challenges the correctness of the standard which the judges have set up, then recourse must be had to a consumer preference test for the final authority as to what the public really likes. Not infrequently, as in judging bread, one may seriously doubt whether the opinions of the so-called experts really represent the preferences of the general public. In such a case recourse can be had to the consumer preference test as the ultimate authority or foundation for the judgment of food flavors and eating qualities.

TYPES OF TEST QUESTIONS

Ideas can be further clarified by considering the different types of questions which may be submitted to the consumer for opinion as to his preference. These cover a wide range of complexity. Three typical examples with increasing complexity are given: First, the identity and difference between the samples is evident to all. The question is, "Which do the consumers prefer?" A choice between chocolate or vanilla ice cream, or between mild and acid cottage cheese are examples.

Second, there is a definite measurable difference known to the manufacturer of the samples but not necessarily to the taster; for

example, bread with 1½ per cent and 2 per cent salt respectively may be submitted or ice cream with 12 per cent and 15 per cent sugar respectively. Samples of known differences in composition can be tested in two different ways. Using the ice cream one can ask, "Which is the sweeter?" By so doing nothing new is learned about the product, because we already know what is in it; but we do learn something about the judges. By decreasing the difference in sugar content one can learn what proportion of people can distinguish differences of five, three, or one per cent at this level. An entirely different question is, "Which do you prefer?" Here one might get a 50-50 vote because the judges could distinguish no difference; or the same vote may result with an entirely different meaning—all judges might perceive the difference in sweetness, but 50 per cent might prefer low sugar and 50 per cent high sugar. Tests should be so framed as to bring out this important difference in meaning.

In the third type of test the difference between samples is not measurable by the manufacturer and is not necessarily noticeable by the taster; in fact there may or may not be any consistent difference between the samples. An example of this is a comparison between bread made with hard winter flour and hard spring flour.

In studying this question there arises at once a welter of questions and doubts. Some of these are as follows:

1. Is there a real difference between these samples which a majority of people can distinguish with certainty?

2. We know that the difference in flavor to be expected from a change of wheat is small at best. On the other hand, difference in flavor to be expected from a change in the degree of fermentation is considerable. If a judge votes for Sample A today and Sample B tomorrow made from a second baking of the two flours, is it not more than possible that his change in vote may be due to accidental variations in the bakings whereby the second bakings got more yeast action? How can we tell?

3. If a vote between Sample A and Sample B shows 55 per cent for A and 45 per cent for B, does it have any significance? If not, is a 60-40 vote significant? If not, how about 75-25? In fact, how can the significance of the vote in a consumer preference test be evaluated?

4. When two samples of foods fairly close together in desirability are submitted to a group representative of the general public, some of the more discriminating will note the difference and show a distinct preference for A. Other equally discriminating individuals may note the difference and show an equally distinct preference for B. Such votes are of importance. We know, however, that a large part

of mankind are not discriminating in their tastes. If a person has two samples submitted to him, however, and is asked for his preference he will nearly always state a preference whether there is any real difference or not. If he were asked to judge a second time between the same two samples differently labelled, he would be just as apt to decide in the opposite direction. We call such a vote a *guess* as distinguished from a definite *choice* which would always be repeated. In a vote between samples A and B, how many votes represent definite choices which would be repeated on a second test and which have therefore definite significance? On the other hand, how many are mere guesses which might be reversed on a second test with the same samples?

When using any piece of scientific apparatus, such as a delicate balance or a huge machine for testing the strength of steel beams, it is customary to test the apparatus—to determine its sensitivity and precision. Likewise before any method of chemical analysis is accepted, someone tests it, and a fair understanding of its precision and limits of accuracy exists. The apparatus and the method are checked against samples of known purity. Replicates are run on the same sample. No reputable chemist would use a piece of apparatus or a new method without some such checks. The greater the errors in a piece of apparatus or in a method, the more important it is to realize clearly what these errors are and make due allowance for them.

USING GROUP TASTERS TO DETERMINE PREFERENCES

If we wish to decide on the public preference for two kinds of ice cream, the scientific instrument to be used is the group tasters. What is the sensitivity of this complicated apparatus? How closely can it duplicate itself on the same pair of samples?

The general *method* for using group tasters comprises first, the selection of the group as representative of the public concerned; and next, obtaining of their opinions through a questionnaire or other means. How reliable is the method? What is the error? At what level do we obtain a significant difference? It is surprising how seldom we ask ourselves these questions or make any clean-cut attempt to obtain an answer. I do not mean attempt to obtain great refinement from an admittedly crude set-up. I mean simply to answer the important questions: "Is the method reliable for the purpose in mind?" "What are the limits of error?"

It is my observation that a great deal of work is done on consumer preference tests in which the results are reported and accepted without any knowledge whatever of their degree of reliability. A great many tests are carried out which yield results that are either

wholly unreliable or are of small value, whereas with a small change in the method they would have yielded results of much greater value. It is my opinion that a critical examination of test methods with a frank recognition of the errors of each step would undoubtedly increase the value of such tests. Such work would be merely applying to consumer preference tests those elements of the scientific method which have done so much to put science, and especially the biological sciences, on a solid foundation.

FUNDAMENTAL ASSUMPTIONS

What are the fundamental assumptions underlying a consumer preference test, then, and how can we estimate the size of the error inherent in each assumption? There are five major assumptions:

First, the judges selected are fairly representative of the whole population concerned. The first step is to define clearly what is meant by "the population concerned." The more this can be narrowed down, the easier is the task. Is the population concerned that of the United States or of a single city? Does it include men and women, children and adults, rich and por? The more people we can exclude, the simpler and cheaper our task.

Making tests on ice cream in Syracuse, we can exclude all of the United States outside of Syracuse; but we must include nearly everyone in Syracuse, for ice cream appeals to all ages, sexes, and classes of society. On the other hand, if we were studying beer, we could exclude children. If studying champagne, we could confine our attention quite fairly to the more wealthy.

If we have a truly random sample of considerable size, then a study of this one sample will indicate to what extent tastes in the city differ as between age, sex, nationality, economic position, etc. Such a sample might consist of one person or family from each city block or every one-hundredth name in the city directory, or it might be a sample of the population which, from analysis, represented a fair proportion of each nationality.

If the sample is not selected at random, however, nothing is known as to whether or not it represents the whole population. Suppose one whole sample consists of workmen in one factory and that they are predominately German. No amount of statistical analysis of this sample of the population will tell anything about the variation in taste in a sample which includes Italians and Jews. On the other hand, three or four different samples from widely different sections of the city, representing all the important groups and showing substantially the same results from each, would give some basis for saying that in the case of the food tested there was no substantial differ-

ence in preference as between sections of the city. In a truly random sample the ordinary methods of statistical analysis will indicate the size of the error to be expected from variations in the population concerned and how large a sample is needed to reduce this source of error to any limits set.

The second assumption is that the preference expressed in the test is the same as would be found if the same foods were eaten under normal conditions. This presents three principal pitfalls to be avoided:

A. The samples are presented under conditions which are grossly artificial or highly unfavorable; for example, samples of some heavy food may be submitted early in the afternoon when the testers are not hungry; the testers may be tired, busy, or uncomfortable at the time of the test; surroundings may be distinctly unpleasant; or a food usually eaten with some other food or a sauce may be submitted without anything to eat with it. The means for avoiding these sources of error are evident.

B. When two samples of food, such as coffee, are submitted for comparison and tasted side by side, there is a distinct tendency for the stronger sample to predominate; the milder one tastes flat. Especially with inexperienced judges a strong sample will often dominate a milder sample, even when the latter is more delicate and fragrant in its flavor. This is a danger which must always be considered. If the testers taste each sample too frequently, their sense organs are especially apt to tire, giving a disadvantage to the milder sample. To check the degree of this effect it is sometimes necessary to submit the samples separately, probably on different days, and simply to ask how much the testers like each one and why.

C. Some foods give a pleasing result when a little is eaten, but we might soon tire of them if we ate more. Further, they might not maintain their popularity if eaten regularly. Yet it is characteristic of some foods, such as coffee and bread, that we eat them every day. Certainly a taste or two is far removed from the conditions of use on every day in the week.

There is no easy way to overcome this possible source of error. In the case of bread, long-time tests have been carried out in institutions by which one group of inmates was allowed to have all the bread of type A that they wished to eat over a period of several weeks. Another similar group was offered bread of type B under the same conditions. The voluntary consumption over a considerable period was thus measured. Somewhat similar tests with bread have been carried out under still more normal conditions, that is, in families. The costs of conducting and checking such extensive tests are evident, but in some cases only a test of this kind will give reliable information.

The third assumption is that the reaction of the same judge to exactly the same food will continue to be the same throughout the period of the test. This assumption is of particular importance when a test is repeated to find out how closely previous results can be duplicated. In such a repetition it is assumed that the same judge tasting the same foods ought to express the same preference between them. Judges vary in their reactions, however, according to whether they are hungry, thirsty, hot, tired, comfortable, etc. Any one might prefer hot tea on a winter day and iced tea in summer. Hence, if a test is to be duplicated, the same conditions should be reproduced as closely as possible. If the second test is carried out under widely different conditions, then it is really a different test rather than a duplicate of the first.

The fourth assumption is that the difference between two samples is greater than the difference between successive lots or batches of the same sample. This is true of any experimental work. In most cases the difference between the samples is so great that this question need not be raised at all. In the case of bread, however, where the difference between samples may be very small and the difference between batches may be large, this question comes up and must be settled.

The final assumption, which has direct practical consequences, is that preferences expressed represent reliable, reproducible decisions and not mere guesses which may easily be reversed on later trials. The fact that when two samples of food are placed before a group of testers nearly all will express some preference, has been previously mentioned. In some cases this will be a definite choice which the same judge will always repeat. In other cases it is just a guess which the tester is as likely as not to reverse on a second trial. Yet the definite choices and the mere guesses look just the same on the records. The real meaning is very different. How can one tell them apart?

The only way to discover the number of guesses is to test the same group with the same samples two or more times, marking the samples differently each time and checking each judge to find how many reverse themselves. The reversals give the number of detected guesses. If a judge is indicating a preference between two samples, however, and if he is merely guessing, he has an exactly even chance to guess the same way on the second test as he did on the first test. In other words, about half of the guessers will, by chance, reverse themselves on the second test. These give the detected guesses. The other half of the guessers will, by chance, pick out the same sample on the second test as they did on the first test. In other words, when there are two samples to choose from, and when the same judges are

tested twice, the total number of guesses is twice the number of guesses detected by actual reversals.

It has generally been the practice to ignore the guesses among the votes in a consumer preference test, but they are an important part of the result obtained. Furthermore, by segregating the guesses the significance of the data is greatly increased. Suppose we examine, for example, the same 100 judges twice with the same two samples of food, A and B, and suppose that these two samples are, like two samples of bread, sufficiently alike so that the difference between them is not obvious. On the first test the results obtained are 60 for Sample A and 40 for Sample B.

On the second test the results are 62 for Sample A and 38 for Sample B. However, on checking the individual votes it is shown that 51 voted for A both times and 34 voted for B both times. Those who reversed themselves, that is, the detected guesses, numbered 15.

From this it is fair to assume that the undetected guesses are just as many as those detected. The 15 undetected guesses are probably divided between A and B roughly in the ratio 51:34; that is, from the 51 repeated votes for A we must subtract $51/51 + 34 \times 15 = 9$ undetected guesses. Similarly we subtract an estimated 6 undetected guesses from the 34 repeated votes for B. The final estimate of the real situation is as follows:

Net definite choices for A	42
Net definite choices for B	28
Guesses	30

Where there are very few guesses we have much more confidence in the results. It is quite common for a check to show that there are more guesses than reliable choices. The number of guesses is a figure of importance as indicating what proportion of the public under consideration can detect any reliable difference between the samples. This point is not necessarily shown by the closeness of the vote. Evidently even when there is an unmistakable difference between the samples 50 per cent of the testers might prefer one and 50 per cent the other.

An example of a vote where testers notice a distinct difference between the two products would be as follows:

Net definite choices for A	55
Net definite choices for B	35
Guesses	10

Such results show a real difference. However, if the vote runs somewhat like the following:

Net definite votes for A	30
Net definite results for B	20
Guesses	50

something important is indicated regarding the two samples, namely, many people cannot tell the difference.

If the same group can be tested with the same two samples three times a more definite line on the number of undetected guesses is secured, because a judge has here only one chance in four to guess the same way each time. Similarly, if there are three or more samples of food instead of two, the ratios are changed but the principle remains the same. A close vote in the preferences between two samples shows that the number of people who prefer one is about the same as the number who prefer the other. However, a large number of guesses shows that many people cannot detect any real difference.

SUMMARY

"Better" when applied to the flavor or eating qualities of human foods can have only one meaning, namely, what the public concerned likes better. This is the ultimate authority.

To determine what the public likes requires preference tests made directly on the public.

The conducting of a test of this sort is full of pitfalls, and results are frequently unsatisfactory if not actually misleading.

To get a clear understanding of the possible errors and to minimize them as far as possible it is helpful to state clearly the fundamental assumptions involved in tests of this kind.

The five fundamental assumptions have been given and discussed.

The importance of guesses in all tests of this kind is pointed out, and means for separating the guesses from the definite choices is described.

In one form or another ideas for "better flavors" must be submitted to the public. Any means which help to make such tests more economical, reliable, and scientific in the best sense of the word are worth while.

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MODERN TRENDS IN FLAVORS[1]

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(Received for publication, May 22, 1937)

In the old days in America, and not so long ago either, flavors were of an exceedingly simple nature; various pot herbs, such as thyme, sage, and coriander, were employed. Spices were generally used as single ingredients. The art of blending flavors was not extensively practiced. The French early took the lead in flavor blends. Today blended or harmonized flavors appear to be the goal of the flavor manufacturer, who is also paying greater attention than ever before to the preservation of the flavor so produced.

The use of spice oils, resins, and blended spices is rapidly taking the place of the whole ground spice. This is especially true in the meat and sausage trades.

Nutrition workers are agreed that a monotonous diet upsets regular digestive processes. Next to a careful selection of food the choice and application of flavor does most to relieve monotony in diet.

PRODUCTION OF BETTER FLAVORS

The modern trend in flavors is along two lines of development which support and strengthen each other. The first of these is a well-defined effort to produce better flavors, since the public is more flavor conscious now than formerly. This is made possible through decided improvements in equipment and in the mechanical aspects of flavor production. In addition, organic research has given the flavor manufacturer a greatly increased number of aromatic chemicals, ranging from the higher alcohols and aldehydes through methyl compounds and methyl derivatives, of sharply defined taste and aroma from which he can produce a great variety of flavor notes and types.

The art of blending or supplying such dominant notes or elusive backgrounds is in line with the modern trend of flavor extracts. It is a well-recognized principle that in minute traces compounds of even objectionable flavor or odor may greatly assist in producing a finished product of superior excellence. The processes of today are more subtle and the results are more satisfactory than ever before.

[1]Read before a symposium on *Flavors in Foods* at the Chapel Hill, North Carolina, meeting of the American Chemical Society, April 12 to 15, 1937.

EFFORTS TO PRESERVE ORIGINAL DELICACY

The other well-defined, trade-flavor trend is that of preserving the original delicacy and palatability of the flavor from the time of production until it reaches the consumer. The growing plant works unceasingly transforming one compound into another, and the process goes on from germination through the production of the fruit and the completion of its life cycle accompanied by destruction of flavor. The flavor manufacturer interrupts the natural processes of deterioration. Oxygen, light, warmth, enzymes, and microörganisms contribute to these rapid changes. One of the tasks of the flavor chemist is to segregate and hold in status quo the more or less unstable flavoring compounds under conditions that will suspend this natural chain of reactions and leave the delicate and delicious flavor at its best.

Oxygen has been called one of the greatest enemies of flavor. As an illustration of its effect upon the flavor of a finished product, the soft-drink industry may be cited. In this field the citrus flavors—orange, lemon, and lime—are important commercially; but because of the presence of unsaturated constituents, such as terpenes, sesquiterpenes, aldehydes, alcohols, and ketones, they are liable to deterioration if oxygen is available. This is particularly true if the juices of these fruits as well as the oils are incorporated in the beverage. The oxygen in the air left in the top of a bottle is sufficient to cause the damage. Machines have been developed for the removal of air in filling bottles, and products are available in which the terpenes have been partially or wholly removed but not, we shall have to admit, without taking something away from the flavors. Instead of a vacuum, an inert gas, such as carbon dioxide or nitrogen, accomplishes the same purpose.

It is doubtless true that in so far as loss of flavor is concerned, deterioration owing to oxidation is just as important as the biochemical changes owing to bacteria, molds, and growths of microörganisms which have long been studied and at least in part controlled. In 1922, hydroquinone was suggested as an antioxidant and since that time scores of other products have been mentioned for this purpose. Those which protect fats and the fixed oils do not necessarily prove beneficial for essential oils because of the difference in composition.

One of the most important of the suggested antioxidants from the standpoint of foods is oat flour, because no exception is taken to its use by food administration officials and because of the reluctance of the public to use foods treated with chemicals. Oat flour has a very marked effect in preventing rancidity of the fixed oils. Rancidity is, of course, the evidence of a considerable series of reactions, the first of which is loss of aroma and flavor. It has given some very interest-

ing results with edible oils, flax seed and castor oils, potato chips, salted nuts, fish products, bacon, flour mixtures, and other products. It has been found also to have a retarding effect upon the oxidation of essential oils of the citrus group, which are especially susceptible to oxidation. Oat flour, therefore, can be used to advantage in emulsions of certain flavoring oils.

In protecting food products from the action of light in glass and other packaging materials the commercial application of available facts has made some progress. There is a definite trend toward the use of dark glass of green or amber color, which bars a portion of the light's rays, in the packing of flavors and essential oils.

In the matter of flavor preservation it is also important that careful attention be paid to the selection of receptacles in which products are processed. The presence of zinc, even in small quantities, has a very deleterious effect on flavor; and contamination is liable to result from it either in alkaline or acid solution because zinc, being amphoteric, is soluble in either. In many cases copper is not desirable because even small quantities of copper act as catalytic agents and promote the degree of oxidation of delicate flavors.

In the choice of receptacles for commercial products consideration should be given to glass-lined containers, stainless steel, solid nickel, Inconel alloy, or some specific material which does not dissolve in the product even to a slight extent.

Among bulk users of extracts there is a tendency towards more concentrated flavors for the purpose of economizing in solvent and delivery cost.

OXIDATION IN RELATION TO OFF-FLAVORS IN MILK AND CERTAIN MILK PRODUCTS [1]

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(Received for publication, May 22, 1937)

Shortly before the turn of the last century bacteria as a cause of disease and as a cause of food decomposition already had been given much attention. It was a natural reaction at that time to suspect that all changes in the flavor of milk and dairy products, after their production, were caused by the growth of bacteria. Butter, because it was stored for long periods, first received more attention than other dairy products. Early investigations of the flavors of storage butter led to the conclusion that many of the changes in flavor were to be attributed to chemical causes, since the low temperatures of storage seemed to preclude the possibility of bacterial growth. Later it was found that milk powders, in which the water content is too low to sustain bacterial growth, also showed changes in flavor which must come from chemical causes.

Recently a certain off-flavor in milk has been attributed to chemical action, since it has been shown that this flavor develops only when bacterial contents are consistently low and that it can be produced by the action of added catalysts. The action of the same catalysts in ice cream has been found to produce a similar off-flavor.

One of the most important of the chemical changes causing off-flavors in all the dairy products just mentioned appears to be oxidation involving butter fat or constituents of milk closely associated with butter fat. There seems to be general agreement among people who work with dairy products that oxidation reactions occurring in butter and in dry milk are concerned primarily with the butter fat.

In the case of milk, opinion is somewhat divided, there being some evidence to indicate that the lecithin of the adsorbed layers on the fat globules is the substance first oxidized, although it is admitted that the butter fat also may be involved.

It will be the purpose here to review some of the experimental work bearing on the relation of oxidation to off-flavors in butter, dry milk, ice cream, and milk.

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[1] Read before a symposium on *Flavors in Foods* at the Chapel Hill, North Carolina, meeting of the American Chemical Society, April 12 to 15, 1937.

OILY AND TALLOWY FLAVORS IN BUTTER

The word "rancidity" usually is employed by the chemist to designate any deterioration of a fat noticeable to the olfactory sense. For purposes of better identification dairy scientists have come to refer to hydrolytic rancidity as "rancid" flavor and to oxidative rancidity as "tallowy" flavor. This paper will be concerned only with oxidative changes. Because of its economic importance the oxidation of the fat in butter has been the subject of extensive research. Results have shown that many factors are concerned in this oxidation under certain commercial conditions.

During the early days of commercial buttermaking in this country it was the common practice to churn sour cream. Sweet cream obtained at the creamery usually was soured by incubation after the addition of a culture, and sour cream either was churned in the condition in which it was received or was neutralized, pasteurized, and then soured by addition of culture followed by incubation. Although it was shown by Patrick, Leighton, and Bisbee (1892) that butter made from sweet cream retained its good flavor in storage better than that from sour cream, the demand for butter of the high aroma and flavor resulting from the proper souring of cream resulted in statements favoring the ripening (souring) of cream for butter making by McKay and Larsen (1906) and Michels (1904). Work by Dyer (1916), Gray and McKay (1906), Mortensen (1924), and Rogers and Gray (1909) has left no doubt that sweet-cream butter has superior keeping quality. Furthermore, Greenbank and Holm (1924) and Wright and Overman (1931) have shown that the presence of acids in butter fat greatly increase its susceptibility to oxidation. Where sour cream must be handled, maximum keeping quality can be promoted by proper neutralization, according to Mortensen (1924); but Hunziker and Hosman (1917) have shown that over-neutralization must be avoided because it favors oxidation. Likewise, Greenbank and Holm (1924) have shown that the addition of alkali to filtered butter fat favors oxidation. The latter workers also have found old fats to be very susceptible to oxidation, probably because of the liberation of acids during aging.

Practically all factory-made butter produced today comes from pasteurized cream because the experiments by Gray and McKay (1906) and Rogers, Thompson, and Keithley (1912) and the experience of the industry have shown that butter from non-pasteurized cream does not keep well, becoming rancid (hydrolytic type) quickly either before or after storage at freezing temperatures. There is lack of agreement as to the correct temperature and time in pasteurization treatment. Mortensen (1924) found 62.8°C.(145°F.) for 30 minutes

favored keeping quality more than 76.7°C.(170°F.) for 20 minutes, and that the latter treatment was preferable to 82.2°C.(180°F.) for 20 minutes. Guthrie, Scheib, and Stark (1936) found butter from cream pasteurized at 74°C.(165.2°F.) for 30 minutes possessed better keeping qualities than that from cream pasteurized at 62.8°C. for 30 minutes, attributing the better effect of the higher temperature to the destruction of oxidases. No improvement in keeping quality was found by White and Campbell (1925), owing to increasing the temperature of pasteurization from 62.8 to 74°C. during a 30-minute period.

Washburn and Dahlberg (1917) and Guthrie, Scheib, and Stark (1936) have shown that salt in butter retards the growth of microörganisms at storage temperatures above freezing, but at lower temperatures it favors the oxidation of the butter fat. Thus butter of low salt content, or unsalted butter, possesses better keeping qualities in cold storage than highly salted butter. In studies of the effects of various storage temperatures Gray and McKay (1906) and Rogers *et al.* (1912) have shown that temperatures of —18°C.(—0.4°F.) or lower are best.

Much valuable information relating to the oxidation of the butter fat in butter has been obtained by the use of a method of measuring the susceptibility of butter fat to oxidation devised by Greenbank and Holm (1925). The amount of oxygen absorbed by a fat kept at high temperature in an atmosphere of this gas is measured by this method. It is found that fats resist oxidation for some time and then absorb oxygen rapidly. The period of resistance to oxidation is called the induction period. The relative susceptibility of fats to oxidation is inversely proportional to the length of the induction period. Tests for peroxides, which always are formed when oils or fats are oxidized, have been used by Greenbank and Holm (1934), Lea (1931), and Peters and Musher (1937) as means of following the progress of oxidation; whereas Stebnitz and Sommer (1936) have used them to detect the end of the induction period. The Kreis test has been found by Holm and Greenbank (1923, 1924) to yield results quantitatively correlated with the amount of oxygen absorbed. The determination of the oxidation-reduction potential has been suggested by Davies (1928) as a means of forecasting the deterioration of butter during storage.

Early studies by Hoft (1909), Kooper (1911), and Marcas and Huyge (1905) revealed that contamination of butter with iron caused the subsequent development of flavors now known to be the result of oxidation. Rogers *et al.* (1913) showed that the lactates and sulfates of copper had a much more intense action than those of iron. Hunziker and Hosman (1917) found that butter stored with either a

rusty nail or copper wire imbedded in it became bleached near the metal and developed a tallowy flavor. They found this to be true also of brass and German silver but not of tin, and were able to produce bleaching and tallowy flavor by the addition of hydroxides of iron and copper. Wright and Overman (1931) showed that butter contaminated with copper had a greatly decreased resistance to oxidation; and Henderson and Roadhouse (1934) demonstrated that the fat of milk contaminated with copper had an increased susceptibility toward oxidation.

It was found by Jensen (1901) that direct sunlight promotes the production of tallowy flavor in butter. It was observed later by Siegfeld (1908) that light also causes a gain in weight, and increases of acidity and of the Reichert-Meissl, saponification and Polenske numbers, but a reduction of the iodine number. Ultra-violet light has been found by Holm, Greenbank, and Deysher (1927) to have a powerful effect in increasing the susceptibility of fats to autoxidation, and the greatest acceleration of the autoxidation of cottonseed oil by the different lights of the spectrum was reported by Greenbank and Holm (1933) to be in the region of the orange band. Even the exposure of milk to direct sunlight reduces the length of the induction period of the butter fat as shown by Henderson and Roadhouse (1934).

Browne (1899) believed atmospheric oxygen to be an important promoter of the oxidation of butter fat in butter at earlier dates, but the general research findings indicate that other factors, particularly acidity and storage temperature, are more important.

Studies of the gases contained in butter, both by Dyer (1916) and by Rogers *et al.* (1913), have shown a reduction of oxygen following storage. Dyer noted a corresponding increase of CO_2 in butter after storage and found that acidity of the cream had a tremendous effect on the rate of O_2 loss and CO_2 production. The storage of butter *in vacuo* does not prevent oxidation and the development of tallowy flavor. Holm, Greenbank, and Deysher (1926) have found evidence of the presence of loosely bound oxygen compounds, termed "moloxides," in butter oil which they believe explain the oxidation of butter *in vacuo*.

About 14 years ago a patented process for the incorporation of CO_2 in butter by displacing a part of the air in the churn with this gas before churning gained widespread attention. Consideration of the process and of available records led Hunziker (1924) to the conclusion that the process had no value in improving the keeping quality of butter. It was shown later by Prucha, Brannon, and Ruehe (1925)

that only by storing butter in an atmosphere of CO_2 in tightly sealed cans was any benefit gained from the use of this gas.

The work of Greenbank and Holm (1924) has shown that the presence of water with butter fat increases its resistance to oxidation. These workers found also that prolonged washing of a butter fat with water at 35 to 40°C.(95 to 104°F.) improves its keeping quality.

Holm and Greenbank (1923, 1924), Lea (1931), and Powick (1923) have devoted considerable research to the discovery of compounds produced in oxidized butter fat and to the mechanism of their formation.

Various substances were found by Greenbank and Holm (1934) to be antioxygenic to butter fat. Hamilton and Olcott (1936), Lea (1935), Moureu and Dufraisse (1922, 1926), and Olcott and Matill (1936a, b) have reported on a number of substances found to be antioxygenic to certain oils other than butter fat, but most of these antioxidants are objectionable for use in a food product. It is interesting to note that the phospholipid, cephalin, a normal constituent of milk and butter, has been found by Olcott and Matill (1936a) to possess mild antioxygenic properties. They found also (1936b) that oils of the germs of various seeds act as antioxidants, and Peters and Musher (1937) have had some success with the use of oat flour as an antioxidant for butter and dry milk. The application of this finding in the butter industry is being promoted by the Musher Foundation.

Cellulose paper and aluminum foil wrappers have been found by Thomsen (1935) to retard oxidized flavors in butter.

OILY AND TALLOWY FLAVORS IN DRY MILK

Dry whole milk (milk powder) might be more extensively used than it is in localities lacking a fluid milk supply were it not for the inherent tendency of the fat in this product to become oxidized with the resulting production of oily and tallowy flavors. One of the indications that the butter fat of dry whole milk is the point of attack in the production of these flavors is the fact, as stated by Coutts (1920), that this product is far more prone to develop such flavors than is dry skim milk.

Atmospheric roller-process powders, made by drying milk in a thin film on a steam-heated drum, have been reported by Coutts to possess keeping qualities superior to spray-process powders, made by atomizing milk in a blast of heated air. Palmer and Dahle (1922) have shown that practically all of the granules of spray-process powders contain a spherical core of air, which is not present in roller-process

powders, and have suggested that this may explain the more rapid development of tallowiness in the former product.

Supplee and Bellis (1925) found that dry milks are prone to absorb moisture from humid air. Dahle and Palmer (1924a) found that dry milks in which the moisture content had been increased from slightly below three per cent to more than four per cent by storage at room temperature in nearly saturated air for several hours became tallowy somewhat sooner in storage than did the original powders. The results of Holm, Wright, and Greenbank (1927), who controlled humidity before storage by the circulation of air of varying humidity through the powders, were somewhat inconsistent but indicated, in general, that the more rapid oxidation of the fat occurred at low moisture contents.

A perceptible decrease in keeping quality as the butter-fat content was increased has been observed by Holm, Greenbank, and Deysher (1925). They found also that such processing procedures as precondensing and homogenizing (1925), and centrifugal clarifying (1926) materially improved the resistance of butter fat in dry milks to oxidation.

The milk usually is given a heat treatment previous to drying. The relative effectiveness of four temperatures, each applied for a 30-minute period, in the order of greatest to least effect in retarding oxidation, has been found by Holm *et al.* (1926) to be 83, 93, 73, and 43°C.(181.4, 199.4, 163.4, and 109.4°F.) respectively.

Storage conditions in relation to oxidation of fat in dry milks have been given attention. The experiments of Dahle and Palmer (1924a) showed that moisture-tight containers made of tin and sealed were superior to waxed paper and cardboard containers, and that temperatures of storage from 4 to 20°C.(39.2 to 68°F.) seem to be equally effective in preventing rapid oxidation; whereas temperatures above 20°C. favor more rapid oxidation. Partial vacuum and atmospheres of CO_2 have been found by Holm, Wright, and Greenbank (1927) to be less effective in preventing oxidation than storage in air, which leads to the conclusion that the inherent keeping quality of the butter fat is more important than the storage atmosphere.

Apparently peroxidase has little or no effect in the oxidation of butter fat in dry milks because this enzyme is destroyed by the factors which favor fat oxidation as shown by Dahle and Palmer (1924b).

OXIDIZED FLAVOR IN MILK

A large number of names have been applied to an off-flavor in milk which apparently is caused by oxidation. Besides the term oxidized, which seems to have been used first by Guthrie *et al.* (1931),

the names cappy, cardboard, papery, pulpy, oily, tallowy, oily-tallowy, oily-stale, metallic, empryreumatic, loamy-astringent, emery, and corrundum have been applied.

Recognition of oxidation as a cause of this flavor has occurred only recently. Its investigation appears to have developed following the studies of Hunziker and coworkers (1925, 1929a, b) which directed attention to the corrosion of the metals in dairy equipment by milk and the solubility and effects of the metals, particularly copper and iron, in milk. Although normal cow's milk contains a small amount of copper, as shown by Dahlberg and Carpenter (1936), Rice and Miscall (1923), and Supplee and Bellis (1922), it has been found by Golding and Feilman (1905), Gondos (1934), Guthrie and Brueckner (1934) Kende (1932), Mohr and Wellm (1936), Ritter (1936), Thurston (1935), Tracy and Ruehe (1931), and others that contamination of the milk with more of this metal tends to cause the off-flavor now commonly called oxidized. Contamination with iron has a similar effect, according to Guthrie *et al.* (1931), Hunziker *et al* (1929a), Kende (1932), Mohr and Wellm (1936), and Tracy and Ruehe (1931); although to a lesser degree, as shown by Greenbank (1936), Hunziker (1925), and Thurston (1935). Investigations by Guthrie and coworkers (1931) and Lea (1931) have indicated that nickel may be a cause of oxidized flavor, but Ritter (1936) has reported that this metal causes an off-flavor not similar to the one caused by copper and iron. No other metals have been found to cause oxidized flavor.

Only within the last few years has there been a general shift from the use of equipment fabricated of tin-coated copper to that made from metals known to have no ill effect on the flavor of the milk. It has been a common observation that the tin-coating on copper equipment erodes with use as the result of the chemical action of milk, alkaline washing solutions, milk stone removers of various types, and of mechanical wearing away caused by the use of brushes and sometimes abrasives. Thus copper surfaces often have been in direct contact with milk. The experiments of Guthrie *et al.* (1931), Hunziker (1925), and others indicate the solution of copper in milk from surfaces of this metal through weight losses of strips of the metal during submersion in milk. Direct determinations carried out by Gebhardt and Sommer (1931b, 1932), Quam (1929) and others also have shown that copper dissolves in milk that is handled in contact with surfaces of the metal. Gebhardt and Sommer (1931a), Wesley (1932) and others have shown that galvanic currents through the milk in equipment made of more than one metal probably cause some solution of copper. Gebhardt and Sommer (1931b) found that air

and oxygen favor solution of copper in milk. As shown by Dahlberg and Carpenter (1936) and Rice and Miscall (1923) oxide-bearing copper surfaces tend to give up more copper to the milk than bright surfaces, which probably explains the greater solution of copper in the first milk passed through a piece of copper equipment than in the milk that follows.

Solution of iron in milk under various conditions has received less attention than copper, probably because its use is limited principally to the manufacture of milk cans which are tin-coated, and also because more iron than copper is required to produce oxidized flavor. Perhaps the formation of oxides on copper-bearing surfaces by the action of chlorine sterilizing rinses may explain the increased solution of copper and the increased tendency toward oxidized flavor development found by Dahlberg and Carpenter (1936) and Tracy and Ruehe (1931) in the first milks passed through such equipment following chlorine sterilization.

Studies of the general suitability of various metals for dairy plant equipment by Guthrie, Roadhouse, and Richardson (1931), Hunziker, Cordes, and Nissen (1929a), Kende (1932), Quam (1929), and Tracy and Ruehe (1931) without doubt have influenced manufacturers and buyers in the rapid change to the use of chromium-nickel-steel, and limited the increase in the use of an aluminum alloy. Furthermore, Ritter (1936) has presented evidence that minute amounts of copper pass through the tin coating into the milk. Those still using tinned copper are more careful to preserve or replace the tin coating than they were some time ago.

Although the solution of copper or iron in milk must be considered an important cause of oxidized flavor, it has been shown by Kende (1932) and Brown, Thurston, and Dustman (1937) that not all milks will develop this flavor following contamination with one of these metals. This fact has given rise to the terms "susceptible" and "non-susceptible," which refer to the susceptibility of milks to the development of oxidized flavor following contamination with copper or iron. Also, it has been shown by Greenbank (1936), Guthrie and Brueckner (1934), Kende, and Brown *et al.*, that some milks may develop oxidized flavor without contamination with either copper or iron and this condition will be referred to as "spontaneous" development of oxidized flavor.

Credit for finding that the susceptibility of milks varies widely, from no susceptibility to spontaneous development of oxidized flavor, seems to belong to Kende. Working in Austria, he was compelled to limit his studies to the winter season because summer milk usually proved to be non-susceptible. Even during the winter the susceptibil-

ity of milks from different cows and from different dairies varied widely. Green feed, usually as pasture, was found by Brown *et al.* (1937) and Greenbank (1936) to have the effect of reducing or eliminating susceptibility. Kende (1932) and Mayer (1931) observed the same effects from feeding fresh hay. Thurston (1935) and Brown *et al.*, found that green feed has more effect on susceptibility at some times than at others, and it has been suggested that the condition of the pasture has much to do with this effect. Brown *et al.*, found also that the degree of susceptibility of the milks of individual cows on the same pasture at the same time may vary considerably.

Brown *et al.*, have shown further that the feeding of tomato juice or lemon juice reduces or eliminates susceptibility, and have explained this effect on the basis of the vitamin C contained therein, since they found that the feeding of .5 gram of ascorbic acid daily greatly reduced susceptibility. Carrots, fed at the rate of five pounds daily, were found by Anderson (1936) to eliminate the susceptibility of the milk of the one cow he experimented with on this factor. Although carrots are not an excellent source of vitamin C, they are a good source.

While vitamin C in the feed of the cow tends to reduce or eliminate susceptibility of the milk to oxidized flavor development, it does not necessarily follow that the vitamin passes into the milk in increased quantities, as experiments by Riddell *et al.* (1936) have shown that the vitamin-C content of milk is not greatly influenced by the ration, and Sharpe (1936) has found little variation of this vitamin owing to the season of the year. At present we can only guess as to the mode of action of vitamin C, although anti-oxygenic properties are indicated by the work of Chilson (1935).

Likewise there seems to be no adequate explanation of the discovery by Prewitt and Parfitt (1935) that rations containing soybean oil, either direct or in the unprocessed beans, have a tendency to produce milk of lowered susceptibility. The work of Tracy, Ramsey, and Ruehe (1933) and of Thurston and coworkers (1936) has shown that homogenization at low and moderate pressures reduces susceptibility; Thurston *et al.*, found that homogenization at high pressures appears to eliminate it. The tallowy flavor found in homogenized milks by Doan (1935) may have been the typical oxidized flavor which, perhaps, would not have developed had higher homogenizing pressures been used. The work of Thurston *et al.*, and of Greenbank (1936) showed that agitation of cold milk reduces or eliminates susceptibility, and Thurston *et al.*, found that freezing and thawing does likewise. Aeration of milk during pasteurization has been reported by Guthrie

et al. (1931) to enhance the tendency for the development of oxidized flavor.

Pasteurization at 62°C.(143.6°F.) for 30 minutes appears to have the effect of increasing susceptibility if contamination with copper occurs after pasteurization, but of reducing susceptibility if contamination occurs before pasteurization, according to the results of Brown *et al.* (1936). However, high-heat treatments such as 85°C. (185°F.) for five minutes have been found by Kende (1932) and Chilson (1935) to reduce or eliminate susceptibility.

The occurrence of oxidized flavor is predominantly in high-grade milk. This fact immediately suggests a relationship between the suppression of bacterial growth and the development of oxidized flavor. Kende (1932) found that oxidized flavor could be prevented by the growth of microörganisms. One of his colleagues, Kertesz (1932), isolated an organism to which he gave the name *Reductobacterium frigidum neutrale.* Cultures of this organism proved very effective in preventing the development of oxidized flavor, yet appeared to have no ill effect on the milk and were said not to be injurious to health. Dry cultures of the organism were prepared and used successfully in additions to milk for the purpose of rendering it non-susceptible. Tracy *et al.* (1933) have shown that the incubation of either raw or pasteurized milk at either 20 or 26.7°C.(68 or 80°F.) for a few hours greatly reduces its susceptibility to oxidized flavor development. The addition of live yeast cells was found to have the same effect. The effects of bacteria are explained by the results of Kende and Tracy *et al.,* on the basis that they bring about a reduction reaction in the milk. In a like manner such reducing substances as hydroquinone and ascorbic acid have been found by Chilson (1935) to reduce or eliminate susceptibility; Greenbank (1936) also has found that ascorbic acid reduced susceptibility.

The relation of the oxidation-reduction potential to the development of oxidized flavor in milk has been studied. Kende (1932) found that milk strongly susceptible to oxidized flavor development had a long reduction time with methylene blue, and stated that increased amounts of reducing substances may be supplied by those feeds tending to render milk non-susceptible. Tracy and coworkers (1933) found that oxidation-reduction potentials of freshly drawn milk had a normal tendency to decrease, but that the addition of copper caused a rise, whereas incubation to cause bacterial growth, or the addition of living yeast cells, caused a decrease. Thurston (1935) showed that copper and iron dissolved in milk caused a rise in potential and the development of oxidized flavor, whereas tin and aluminum caused a decrease without oxidized flavor development. Aeration with oxy-

gen, however, produced a marked rise of the potential without causing oxidized flavor. This effect of oxygen and a variation in the potential at which oxidized flavor developed led him to believe that the potential was not a reliable indicator of susceptibility. Webb and Hileman (1937), however, found that the potential was roughly proportional to the percentage of individual milks in the mixture which became oxidized, but found no relation between the potential and the spontaneous development of oxidized flavor in the milks from individual cows. Greenbank (1936) found that a susceptible milk showed a marked rise of potential on the addition of copper, but no such rise owing to similar treatment of a non-susceptible milk. He suggested that the "poising" action in the latter sample was caused by the unknown substance which inhibits occurrence of the flavor.

Work on oxidized flavor has not revealed the constituent of milk affected when this flavor occurs. The fact that when milk is fractioned as in the ordinary manufacturing procedures, such as separating and churning, the flavor remains strong in the portions containing butter fat, led at first to the belief that the butter fat itself is oxidized. Without doubt the general knowledge that the oxidation of the fat of butter causes an oily or a tallowy flavor, as discussed in the first part of this paper, had considerable influence in the rise of this belief. There is evidence now that some constituent other than fat is affected. Thus it was found by Henderson and Roadhouse (1934) that the fat of milk carrying oxidized flavor had passed only a fraction of its oxygen-uptake induction period, which indicates little likelihood that the oxidation of the milk fat had progressed far enough to become oxidized or to impart oxidized flavor. Furthermore Greenbank (1936) has remarked that, ". . . this oxidation occurs at 5°C. in an atmosphere containing approximately 2.5 per cent oxygen. This fact would seem to exclude from consideration such constituents as fat, casein, lactose and albumin which are relatively stable."

The opinion has been expressed by Mohr and Wellm (1936) and by Thurston *et al.* (1935) that the oxidized flavor of milk differs distinctly from the tallowy flavor of butter, and the last named workers found that the off-flavor of skim milk in which tallowy butter fat has been dispersed differed definitely from true oxidized flavor.

Thurston and coworkers (1935) have postulated that the lecithin of the adsorbed layers on the fat globules is the substance affected, on the basis of their finding that the addition to milk of the product referred to as lecithin, which was extracted from dry buttermilk and which became oxidized during handling, gave the milk a typical oxidized flavor. It was found also that the off-flavor followed the

butter-fat portion of milk during separation and churning, but that when the adsorbed films were removed from the butter fat only a slight oxidized flavor persisted. In further work by Thurston *et al.* (1936) agitation of cold milk, which was found to reduce or eliminate susceptibility, was shown to cause a movement of a portion of the phospholipids from the adsorbed layers on the fat globules to the plasma in which the off-flavor apparently cannot develop. Freezing and thawing had a similar effect. Both agitation, and freezing followed by thawing, caused considerable coalescence of the fat globules, a condition known to cause movement of phospholipids from the fat-globule layers to the plasma. Not all the lecithin is moved in this way, however, so that these findings must be considered as circumstances to be more fully explained by further work, especially since homogenization, which causes division rather than coalescence of fat globules, also retards or eliminates susceptibility.

Greenbank (1936) has suggested that if lecithin were the constituent oxidized, all milk samples should develop the flavor since all milks contain lecithin. This is contradicted by several conditions, one being that although it is not considered that homogenization at high pressure removes any constituent from milk, yet susceptible milks subjected to this process become non-susceptible.

An oxidizing enzyme may be concerned in the development of oxidized flavor. On the basis that milk heated to 85°C.(185°F.) for five minutes became non-susceptible, Kende (1932) postulated that an enzyme, which he called "oleinase," is necessary for the oxidation. Studies by Majer (1932) and Greenbank (1936), as well as some of our own (unpublished), have shown that although high-heat treatment often renders susceptible milks non-susceptible, it does not do so in every case. According to Greenbank, additional copper is necessary to cause oxidized flavor in the high-heated samples. Perhaps the enzyme found by Sharpe (1936) to cause the oxidation of vitamin C in milk also may be concerned in the development of oxidized flavor. This suggestion is especially interesting in view of the findings that ascorbic acid added to the milk or to the feed of the cow acts as an inhibitor of oxidized flavor development.

Exposure of milk to light, especially direct sunlight, causes an off-flavor which probably is the result of an oxidation. Hammer and Cordes (1920) found that a tallowy flavor, accompanied by a burnt flavor, developed in milk following its exposure to sunlight in plain glass bottles, and that the development of the tallowy flavor was hastened by the presence of metals known to be in the milk; whereas these metals had nothing to do with the development of the burnt flavor which could be caused in skim milk as well as in whole milk.

Apparently the burnt flavor develops in the plasma, whereas the tallowy flavor occurs in the butter fat or its adsorbed layers. Work by Frazier (1928) and Whitehead (1931) also has shown that an off-flavor may be caused in milk by exposure to light. There is some question as to whether metallic contamination was adequately controlled in these experiments with light, and for this reason it is difficult to draw conclusions regarding the nature of its effect.

The exposure of milk to ultra-violet light in the process of irradiation for the purpose of increasing vitamin-D content has been reported to cause some unusual flavors, but these flavors have been said by Weckel *et al.* (1934) to develop in the proteins of milk rather than in the butter fat. It is likely that this flavor, as well as that caused by sunlight, is the result of an oxidation.

STALE-METALLIC FLAVOR IN ICE CREAM

A disagreeable flavor called "stale-metallic" or "cardboard" is found frequently in strawberry ice cream. Dahle and Folkers (1932), on scoring several hundred samples of strawberry ice cream which had been held frozen for two months, noticed that no cardboard flavor developed in samples in which dry skim milks had furnished the serum solids concentrate, but the flavor did occur in most samples containing condensed skim milk. As most of the condensed milks were evaporated in vacuum pans constructed of copper, the relation of copper to this off-flavor was indicated.

Tracy and coworkers (1934) conducted a comprehensive study of the causes of this flavor. Contamination of the ice cream with copper was found to be the principal contributing cause since the flavor failed to develop in ice creams not contaminated with copper. The fact that the defect decreased progressively with increased temperature treatments of the berries would indicate that an enzyme in the fruit aids in the catalysis; and the fact that the flavor still occurred at a reduced intensity may justly be ascribed to the effect of copper on the milk products, since copper-contaminated ice creams were shown to develop the stale-metallic flavor in the absence of the fruit. Besides strawberries, it has been found that oranges, lemons, pineapples, apples, potatoes, peaches, and apricots favor occurrence of stale-metallic flavor when used in amounts of from three to 10 per cent of the amount of ice cream flavored.

The oxidation-reduction potential was found to move toward the reduction side when 10 per cent of strawberries were added to non-copper-contaminated mix, but the addition of copper caused a rise of potential and a coincident development of stale-metallic flavor. The potential reduction was directly proportional to the amounts of berries

added, and when 15 per cent or more of the fruit was added, the off-flavor did not develop. Tracy (1936) reported later that an Eh of .433 to .438 appears to be necessary for the appearance of an oxidized flavor in vanilla ice cream.

The fact that contamination of the ice cream with copper did not cause stale-metallic flavor in ice creams containing practically no butter fat, and that the intensity of the flavor becomes greater as the percentage of fat is increased, indicates that an oxidation of the fat or of substances closely associated with it occurs.

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MEASURING FOOD FLAVORS[1]

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(Received for publication, May 22, 1937)

Flavor has been too much ignored by science. It should appeal to chemists particularly, for smelling and tasting are direct chemical sensing. These senses are distinctly intimate and personal but that should be the very reason for studying them intensively. Through better flavor understanding and appreciation, chemists can contribute directly to better living by further improvement and preservation of flavor in the foods with which they are concerned.

A first step in flavor appreciation and control is flavor measurement—the kind of quantitative investigation scientists demand, which distinguishes sciences from arts. This paper deals with the first step—flavor measurement.

In this general presentation of the elements of present-day flavor evaluation, both theoretical and practical aspects are considered. It is a sort of survey for later reference, when the subject will be better covered than is possible today. The theoretical part is concerned with the individual animal senses involved in flavor perception and, as far as is known, the components of unit nerve-types of each of these. It is also concerned with thresholds of sensitivity and the relation between stimulation and sensation. The practical part has to do with laboratory methods as well as a brief survey of the technique of professional tasters. A number of important food and beverage concerns have coöperated in this part of the work and their coöperation is gratefully acknowledged. This paper concludes with a general analysis and discussion of the subject and some specific suggestions and recommendations.

WHAT IS FLAVOR?

Flavor has been defined as that property of a food or beverage that makes it excite the senses of taste and smell. This definition, on examination, is found to be somewhat narrow, for other factors than taste and smell contribute directly to flavor. Menthol, for instance, produces a strong feeling of coolness in mouth and nose, and this coolness is a particularly important part of the flavor of peppermint. The pungency of mustard, pepper, and other spices is likewise important and is part of their flavor; this is also true of the

[1] Read before a symposium on *Flavors in Foods* at the Chapel Hill, North Carolina, meeting of the American Chemical Society, April 12 to 15, 1937.

feeling of the smoothness or graininess of milled materials, such as peanut butter or chocolate. Oiliness is perhaps in large part a feeling. Flavor, therefore, must take in the sense of feeling in the mouth and nose.

We may properly rule out appeal to eye and ear, although both of these unquestionably have the power to stimulate appetite and may create the desire to eat certain products. Commercially, a raspberry jelly has to have exactly the right shade and intensity of red color to go with the flavor. Appropriate color is to all of us a symbol for flavor and belongs with every finished article. Sound may stimulate appetite. Crackling in milk may make a cereal interesting. Over the radio the explosion accompanying the opening of a bottle, the tinkling of ice in glasses, or the sizzling effervescence of a beverage can create a very real thirst. It would be too far-fetched, however, to include either color or sound as a physical part of flavor.

Our appreciation of flavor is much involved with psychological matters, such as our experiences and inhibitions. This is well illustrated by a sage reminiscence of the Roman poet, Horace, made two thousand years ago:

> "If your steward is away, or the sea is too rough for fishing, cravings within you will be quieted with a little bread and salt. How does this come about? Most of the pleasure is in yourself, not in the odor of expensive food. Hard exercise is the best sauce. The fat sallow gormand cannot enjoy oysters, scar, or imported lagois."

One of the first things a chemist has to learn to do, while evaluating flavors, is to smell or taste objectively for what a thing is apart from his whole personal background of associations between odors and experiences; and there are many. Odor and taste strike deeply into one's consciousness and are sharply remembered and keenly associated. One can acquire the ability to observe objectively, however, at least during working hours.

Since mere sniffing of an aroma does not as a rule give one all of a flavor, whereas "tasting" in the mouth does, flavor may be defined as that property of foods and beverages that causes sensory stimulation when they are taken into the mouth. In its old and nontechnical sense the word "tasting" describes the complete detection of flavor. Much is known about the separate types of stimulation which give the sensation of flavor, but no system of coördinating them has been worked out that encompasses the whole subject. We will, for convenience in this paper, divide flavor into three categories and consider two of these, the technical entities of taste and smell, in some detail and "touch" in an introductory manner. If the sub-

division or the treatment is not complete or entirely correct, at least the subject will have been mapped out.

DISTINCTIONS BETWEEN THE SENSES OF TASTE AND SMELL

Taste and smell are quite separate and distinct. In the laboratory the stimuli for these two senses often can be separated by steam-distilling a given material. In a typical instance, such as in a fruit juice, the smell stimulants will go almost entirely into the distillate and the taste ingredients will remain in the flask.

Sometimes, as with coffee, solvent extraction may serve to separate the two kinds of stimuli; for odor stimulants are nearly all oily and are soluble in chloroform or petroleum fractions; whereas taste ingredients are always more or less soluble in water and are frequently not soluble in other solvents.

In some instances an essential oil, such as oil of cassia or oil of anise, may have a sweet taste; or an acid, such as the acetic acid in vinegar, will have not only a smell but a taste as well. These cases are not typical and do not prevent consideration of the senses as separate entities.

A quick practical way to distinguish between smell and taste is to sniff the substance. What is smelled is usually only odor. Put some of the substance on the tongue while the nose is held or plugged. Then the sensations are principally those of taste. Unless the nose is well-plugged, however, some aroma may enter the nose from the throat and smelling will be done under the very favorable conditions of body temperature and high humidity.

From the quantitative standpoint tasting, according to Crocker (1935), is a "milligram" sense, whereas smelling often involves millionths of a gram or less. Smelling is comparable with spectrum analysis in the smallness of the amount of material needed to give a distinct indication. By comparison the sense of taste is very insensitive. Odors reach the nerve-ends of the smelling area in the nose in vapor form greatly diluted by a mass of air. Tastes are presented to the taste buds on the tongue in watery solution. We sometimes think of tasting dry substances or oily liquids but it has been well demonstrated that in the majority of cases, and perhaps in all, only substances actually in solution in the saliva or other watery liquid can be tasted. It is interesting to note that sweet, sour, and salty tasting substances are usually freely soluble in water, whereas bitter-tasting substances are frequently of very limited solubility.

The chemist should be tremendously interested in these truly chemical senses of taste and smell. In the case of odor, molecules are able to impress themselves directly on the nerve-ends, and they

do so in such distinctive manner for the different types of odors that this direct type of impression should be valued far more highly than it is. Whether we shall ultimately find that the molecules excite the nerve-ends by their electrical fields, as postulated by Arno Mueller's (1936) "dipole" theory, or by an effect communicated from the vibrations of the atoms within the molecule, as postulated by Sir Malcolm Dyson (1937), the subject at least is highly important and interesting. We may properly have the greatest admiration for the animal body, which is able to perceive directly the electromagnetic vibrations of heat and light, the pull of gravitation, the air vibrations of sound, also many molecular forces or vibrations, and direct effects from certain ions. Let us sniff and taste to learn more about chemistry.

ODOR AS A PHYSICAL ENTITY

The subject of odor is only recently receiving a measure of scientific attention. While many people feel that smell is conveyed to the brain by only a limited number of components, there is no good agreement as to how many there are or what these may be. Various systems of odor classification have been devised, including the one by Crocker and Henderson (1927). This system, which was based on comparison of the odor sensations given by some hundreds of substances, postulates that there are only four kinds of smell nerves corresponding to the components fragrant, acid, burnt, and caprylic.

The individuality of the vast number of recognizably different odor sensations appears to depend on the relative degrees of stimulation of the four components. A number system proposed describes this type of relationship. It specifies any odor qualitatively as a four-digit number with eight as the maximum possible intensity for any one component. An example of its use is the symbol 3803 for the odor of acetic acid, where three indicates three degrees of a possible eight of fragrance, all eight of sourness, no burnt, and three out of a possible eight of caprylic (goaty).

By careful smelling from similar small beakers equally filled, and using the same nostril for both parts of each comparison a good operator can detect odor-strength differences of perhaps 15 or 20 per cent. By matching against a series, results may be even better. While most strong odors are best compared by the unaided nose, from blotters or beakers, the odors of weak solutions are most conveniently measured for intensity with the nose extended by means of one or the other of several types of tubes now in use by water-purification engineers, described by Fair (1933). These probosces conveniently reach into flasks, where the odor is at its optimum value in equilibrium with the sample of water being tested. With a Fair Dilution Osmo-

scope, which dilutes the air under test by two-to-one ratios with plain air down to one thirty-second intensity, it is usually possible to find a particular dilution where the odor just vanishes, at least over weak-smelling liquids. These two-to-one steps are close enough together for most engineering work, and yet two-to-one odor differences are plainly detectable by relatively untrained workers.

It has been pointed out by Fair (1933) that for smelling, as for hearing, the intensity of sensation varies as the logarithm of the intensity of the stimulus. For sounds the "decibel" system, described by Nilson and Horning (1935), has proved very convenient and practical. In that system the logarithm of the sound energy is the basis of the scale. A 10-decibel sound-intensity difference corresponds to a sound-energy ratio of 10 to 1, a 20-decibel difference to 100 to 1, and so on. The decibel values for a quiet office may be 35, and for a noisy one 55, for a riveter, 100, and for an airplane motor, 110. Pain nerves may be activated when the sound intensity is much above 100 decibels. A difference of one decibel in intensity is detectable to trained acute ears, and one of three decibels to untrained ordinary listeners. This same sort of logarithmic relationship of energy to effect, holding for odor as it does for sound, suggests the type of scale that may be used when a smell-measuring device is discovered.

The Dyson theory of odor production, briefly referred to in a previous section, is based upon atomic vibrations within the molecule. These vibrations are assumed to be communicated directly to the nerve-ends which are stimulated thereby. Dyson is convinced that the particular vibrations causing odor are the identical ones that cause the "Raman effect" and considers that those vibrations causing "Raman shifts" of from 1,400 to 3,400 units account for all distinctive odors. The Raman spectra customarily are obtained from substances in the liquid state, whereas smelling is done in gaseous form, where the molecules are diluted with a very large proportion of air and some moisture; and this concentration difference must surely introduce important factors. Even so, apparently only those substances that produce a distinctive 2,500-unit line in the liquid spectrum smell like mercaptans and only those with a 3,300 line have the pyridine type of odor, etc., for a great variety of chemical types. The Raman spectrum is not easily measured, calls for a mass of liquid, and is otherwise inconvenient. This theory of odor from atomic vibration is very attractive, however, and may suggest some type of electric cell, which will respond as the nerve-ends appear to, that may yet operate the smell meter that is so greatly desired.

While on this subject of vibrations and spectra it may not be amiss to note that the one octave of visible spectrum affects the eyes through

four sets of nerve-ends. Thus several thousand fine gradations of color can register on the brain through the medium of the four kinds of nerves that make up the optic nerve bundle. The Dyson theory of odor calls for only about one and one-half octaves of vibration for all odor types. It is conceivable that a comparatively few nerve types should serve to convey all the needed information for odor as they do for color.

TASTE AS A PHYSICAL ENTITY

Physiologists have given much study to the sense of taste and are agreed that there are four fundamental taste sensations: sweetness, sourness, saltiness, and bitterness. Some workers believe that there are other minor but nevertheless fundamental tastes, such as the "limey" taste of alkalies, while others ascribe this so-called alkaline taste to desquamation of the tongue with consequent feeling sensations.

In a study of the "glutamic taste," made by Crocker and Henderson (1932), "thresholds" of concentration were set up which appear to be typical for average good tasters. Threshold values (that for bitter was recently revised) are as follows:

Taken as teaspoon sips (4 c.c.)	Sweetness = cane sugar	.68%
	Sourness = tartaric acid	.019%
	Saltiness = sodium chloride c.p.	.18%
	Bitterness = caffeine U.S.P.	.0090%

Any tasty product which is liquid or which can be converted into liquid form may be diluted until the distinctive taste is just detectable. Then the dilution factor gives the number of thresholds of total taste originally present if the taster has normal sensitivity.

If only a single taste constituent is present, the problem of matching for equal taste intensity is simple. It is when several taste factors are present simultaneously that ingenuity comes into play. Usually a titration to measure the acid present, and perhaps a chloride determination to measure the salt present, will help materially in finding a match. Specific gravity, refractive index, or a total solids determination may measure the sugar present. If this match is made well, the several components present should disappear on dilution about as they do in the solution under test.

A number of laboratory tests have indicated that on taste comparisons, using half-teaspoon amounts of solutions, most people can readily distinguish strength differences of about ten per cent. Persons of better than average discrimination may make only one or two errors in ten comparisons with as little as six per cent strength difference. This applies to the components—sour, sweet, and salty—in the range of from two to five "thresholds" of concentration. Bitterness is somewhat harder to compare accurately, owing in part to

the awkward position of the bitter-tasting area at the back of the tongue and perhaps also to interference by the feeling of astringency which accompanies the bitterness of many substances. This needs investigation.

When two or more taste components are present together, training is necessary in order to distinguish between them. Even with training, salt and sour may appear to reinforce one another. The best checking is done against actual mixtures of known composition similar to the one under test. Comparison is then simple and sensitive to small differences.

It has been noted by various workers that certain very sweet organic compounds frequently have higher homologs that are bitter, and there are plenty of instances of pure compounds with simultaneous sweetness and bitterness. The simple monovalent salts of the alkali metals cover the range from salt to bitter in similar fashion. Compounds of lithium and sodium tend to be merely salty, whereas those of potassium are salty and more or less bitter, and those of rubidium are strongly bitter. Likewise, chlorides as well as thiocyanates and acetates tend to taste salty, bromides slightly bitter, and iodides bitter. In these cases the salt to bitter transition is gradual and overlapping. It certainly implies similarity in the taste-bud mechanisms for the several taste elements.

An electrical device appeared some time ago that measured the sourness of an apple or other fruit by the deflection of a meter which was actuated by the potential produced when two dissimilar electrodes were plunged into the fruit. Salinometers have been produced to measure saltiness through its electrical conductivity, where conflicting ions are known not to be present. Apparently no instruments are yet available for detecting sweetness or bitterness.

AN EXAMPLE OF TASTE INTENSITY MEASUREMENT

Some time ago our laboratory had occasion to determine the relative sweetness of several sugars. The simple technique used is applicable to other materials and taste-elements and is here presented, in brief, for its suggestions.

The sugars studied were as follows:

A. A dry sugar, rich in levulose.
B. A processed syrup of 76% solids content.
C. "Cerelose No. 50, anhydrous," a commercially pure dextrose.
S. Cane sugar of confectioners' medium-granulated grade. This high-grade sugar, our standard for comparison, was coarser than ordinary household grade and made clear, pale-colored solutions.

The water for making the solutions presented a problem at the time. Distilled water was too rank-tasting, even when aerated, and Cambridge tap water at that time was rich in algae taste and variable from day to day. We decided upon bottled "Poland Water" (well known and readily available, especially for work that might be repeated later) and found this water suitable.

The sugars were made up into solutions, by even percentages, from one to eight per cent. The tasting was done by two operators, working independently, taking small sips from one-ounce (30-c.c.) glasses, code-marked by a person not doing the tasting. (In all similar work now being done tasting is done from teaspoons, about 4 c.c. per taste, instead of by sipping. This insures equal-volume tastes.) The first series was to rate roughly. It showed that A was sweeter than S, B was about the same, and C was much less sweet. Second and third series were then run, continuing the blind coding and using amounts differing by one-half per cent in concentration in the indicated useful ranges, and these results were tabulated. Data obtained are shown (Chart 1), using the "S" line, from the origin to 100 on the right, as the basis against which all other concentrations of equal sweetness were plotted. For instance, two and one-half per cent A was not distinguishable from three per cent S, three per cent A from three and one-half per cent S, five per cent C from three per cent S, etc. The plot shows by its intercept on the right the relative sweetness of A, B, and C, compared with the standard S. By this technique the individual observational probable errors of perhaps ten per cent were conveniently reduced through the operation of plotting, by the factor of the square root of the number of observations at all concentrations, by both observers.

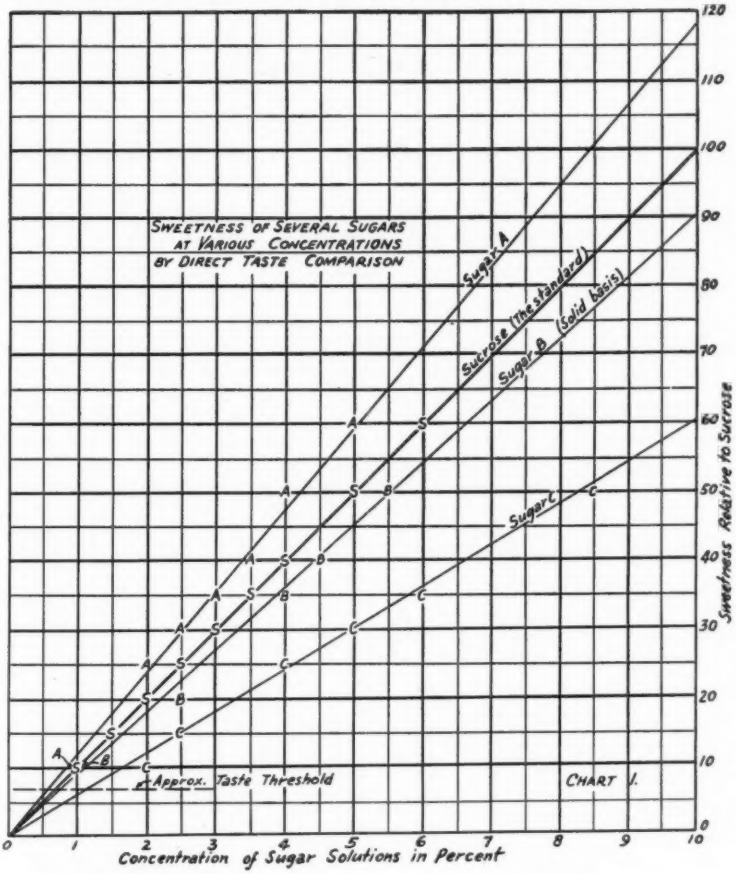
FEELING AS A COMPONENT OF FLAVOR

It has already been cited that menthol and oil of peppermint depend on the sensation of coolness for their flavor and that the pungency of spices is of considerable importance. Pepper, mustard, ginger, or tobacco that doesn't bite the tongue is deficient in flavor. Astringency of tea and other herbs and "bitters" is an important flavor characteristic.

The touch sensations of coolness, warmth, contact, and pain are markedly involved in certain flavors and deserve a measure of attention that they appear never to have received. Such a study should seek the basis of that fine comfortable feeling that the Germans have named "Vollmündigkeit." "Mouthfulness" is the effect and "body" is the condition that produces it, but what are the sensation factors involved?

INDUSTRIAL FLAVOR EVALUATION

In a recent court case in England one of the witnesses, an analytical chemist, claimed to possess a "chemist's nose." This elicited a lively editorial in *The Times* of London (1937), in which the editor



quoted from Shakespeare and others, through Coleridge to Chesterton. The editorial ends with this sombre reflection:

> "There is no question but that the mere possession of knowledge gives pleasure to truly intellectual persons. But sometimes a heavy price must be paid. Imagine our chemist, with his nose, a guest at our dinner table. He would cause more anxiety than a tea-taster at tea. He is almost certain to discern too much for his own enjoyment, and, however good his manners, for his host's peace of mind. He raises his glass

> to his lips; he drinks, but without enthusiasm. His nose has already analyzed the bouquet into constituents that prove our wine-merchant a fool or a rogue. So too with the coffee and the cigars. Unhappy the man who knows too much, unless he is content with knowledge for its own sake. The bearer of a chemist's nose must suffer for his lonely greatness. The common run of the fallen sons of Eve will be happier with their measure of noselessness than they could be with the nose of the chemist, or of the dog."

Apparently this editor was unduly concerned about the disadvantages of keen flavor preception and overlooked the joys. Among the many professional tasters with whom the writer has become acquainted, nearly all are thoroughly jolly and human and enjoy life. If anything, they enjoy the banquet table a bit too much for their own good. Too often they feel as did the essayist Montaigne when he wrote:

> "I should have been glad, in order to form an opinion, to have shared the art of those cooks who are able to blend foreign odours with the savour of their viands, as was particularly remarked in the service of the King of Tunis, who in our time landed at Naples to confer with the Emperor Charles. They stuffed his meats with odoriferous drugs with such extravagance that the cost of dressing a peacock and two pheasants after their fashion amounted to a hundred ducats; and when they were carved up, not only the banqueting-hall, but all the rooms in his palace, and even the neighboring houses, were filled with very sweet fumes, which did not vanish for some time after."

He may, in addition, have an appreciation of the influence of history on food quality as did Horace, when he wrote:

> "The epicure who would avoid tasteless meat should see to it that the wild boar, which bends the round platter double, is brought from Umbria and is fattened on acorns. A boar from Laurentum is worthless, because it has been fed on sedges and rushes."

Many professional tasters are people with only normal taste and odor sensitivities who happened, as boys, to take jobs in tea or coffee blending plants, or apprenticed themselves to chefs. Long years of practice at their art has not sharpened their sensitivities to any appreciable degree. Indeed, some may be the possessors of slightly less than normal sensitivity and yet be very valuable tasters. The art of tasting is one of learning how to concentrate on the indications of palate and nose, and particularly of learning what to look for as the "critical" factor in any article with which one is working. This does not underrate the ability of the professional taster, whatever his line.

but merely focuses attention on the real factor of his value—fine discrimination. Long and careful training and a specialized education are required to attain the necessary proficiency in this kind of work.

In general, the taster looks for one single quality at a time; for example, both tea and coffee tasters look for "body," which may physically correspond to the chemist's term "total extractives," as one chemically trained taster suggested. They also look for a property called "acidity," which has little or no connection with the chemical term of the same name. The tasters' "acidity" appears to be the distinctive flavor of the article under examination. A characteristic of their work is that they go over their tasting again and again to be sure of the findings and to check with standards which are in the actual tasting lineup. With these precautions they check themselves and others amazingly well.

A feature of tea and coffee work is blending. The tasters check the incoming samples according to how closely they come to a particular component of the blend. It is now well recognized that a high bodied lot is almost necessarily low in acidity, and vice versa. The two properties are attained at different altitudes or under different weather or cultivation conditions. Blends are made which combine the right degrees of body and acidity and other lesser properties, and these blends are in general vastly more satisfactory to the user than is any single "pure" sample. Further, they may be maintained, year in and year out, with fine precision. Blending has made the cigarette popular and is today being used for getting good balance of properties for whisky and certain varieties of sherry and champagne. It would seem to be desirable for application to our domestic wines, which appear to be excessively variable from one purchase to the next.

For butter there is a well-worked-out scoring system in regular use, with plus and minus values for departure from an easily attainable "90" condition. The trade handles butter scored as 87 or 93 or some other score as a definite commodity. Some buyers and users may prefer an 87 butter to a 93, some prefer strong-tasting milk or cream to that with a neutral flavor, but such is their choice. By means of the scoring system there is segregation by quality, and those who like off-flavors can get them just as surely as those of more refined sensibilities can secure their choice.

Flavor houses test essential oils, such as clove and wintergreen, that are to be used in flavoring by an elaborate technique. The blotter test, wherein a drop or two of the oil is allowed to evaporate from a narrow strip of filter paper, is used to show up, on nasal

inspection, odor intensity and any unusual odor character in any part of the evaporation period of the oil. Usually a normal oil is run simultaneously for check on both character and intensity. These oils, or the concentrates of natural fruits, are then tested for taste from a dilute emulsion or solution in strong sugar syrup and, if possible, in the dessert or beverage or other product in which the flavor is to be used. In addition a few drops of the oil may be dissolved in alcohol and added to hot water to inspect the odor when it is strong. Spice inspectors depend a great deal on the appearance of the unground article, but also study the aroma of a strong water decoction. Organoleptic assay of red pepper has been described by Munch (1929).

Recently, an article by Willkie, Birnff, and Althousen (1937) was published on the assay of strength of the flavoring raw materials entering into gin manufacture to assure greater uniformity of flavor than had been usual in the product. The juniper berries, cardamon seeds, and other ingredients are evaluated in terms of the percentage of volatile oil and its refractive index and other chemical and physical values which have been found by trial to run parallel to the flavor value of the respective samples. This method, which appears to be general for the industry, does not measure flavor itself but those quantities that strongly influence flavor in gin.

An important corollary to the possession of moderate threshold sensitivity by professional food judges and inspectors is that a large proportion of the public is very sensitive to flavor. This high sensitivity, combined with a low average of training, makes for many strange results. Users may often be misled by incidental factors that are unconsciously lumped in with flavor, such as color, texture, the authority of brand names, or direct suggestions.

Many industries judge the flavors of their line by the jury method. In some cases the jury is made up of well-trained persons able to judge likely "customer acceptance," and good decisions are obtained. In many instances this is absolute judgment without standards for comparison. In other cases the goods are broadcast to a non-resident, untrained group of the public which decides that the "A" is liked better than the "B" or the reverse, because of what they believe is flavor but which in reality is perhaps some feature of color or feel.

A profession has grown up for the intelligent testing of public taste. The attempt is made to get reactions on only a single variable at a time, so that real preferences will be obtained, on the assumption that all favorable effects are additive, which assumption may or may not be checked. Use of this type of service may save much guesswork in introducing new articles or in changing existing ones.

DISCUSSION

Producers of advertised food products naturally value the good opinion of the public. The attempt of each canner of foods is to have in his product every bit of the quality of the fresh fruit or vegetable, when at its best. This is very different in principle from the viewpoint of the perfumer, obtained from special correspondence (1937), who has learned that he cannot make big sales on accurate duplications of flower perfumes alone but must create *idealized* flowers, groups of flowers, or now, the odors of whole woodland scenes, the "Breath of Spring," or something equally intricate. Perhaps the canner is too serious in his attempt at naturalness, which is not often preservable in entirety.

Most industrial and hotel chefs rely exclusively upon the internal standard—their own judgment of what is "right." Certainly the finely discriminating tastes of these judges are better by far than the tastes of the majority of users of food. Nevertheless, this method of judging appears to fall short of what could be obtained by the use of physical standards, even those of their own preparation. Most good musicians have scant respect for anyone's dependence on a supposed "absolute" ability to judge pitch, and themselves insist on "sounding the A." Where possible, physical standards should be used to check and to educate the "internal" standard to make it still more accurate. The term "measure" has nearly a dozen specific shades of meaning in the dictionary but each one has the underlying thought of *comparison* of the quantity in question with some kind of standard. The carpenter, no matter how good his eye, has learned that a rule is even better. The principle, at least, must hold for flavors, for appraisal of quality implies measurement wherever possible.

In much industrial flavor evaluation the first impressions are gained through smelling. The odor is frequently the means for classifying or rough-judging the sample. Then the taste, in its broad sense involving all that is done in the mouth, is used for the accurate judgment. The judgment itself is not for the separate sensation elements —sweet, salty, sour, and bitter—but for substances of overall properties known or believed to be present. For instance, a processor will taste a mayonnaise for its vinegar, its spice, and its smoothness of texture; and a coffee cupper will taste for "body" and "acidity." This is not tasting for the ultimate sensation elements. Whether an even more analytical approach than is now general would obtain additional speed or accuracy over the present system is not known. It might be well, however, in the training of flavor judges, to familiarize them with the principle of the more classical *sensation* detection as against the more industrial *ingredient* detection. They can later use the one they

deem most useful, but sometimes that might be the now neglected sensation analysis, particularly if they are judges of varieties of products.

Professional tasters are pioneers and prophets who have long been working out practical methods that they are able to use as individuals, or as crafts, but it is now time for sympathetic scientists to join in the work. They should interpret, analyze, try to place flavor measurement on a more exact basis, and advance it with the aid of instruments and adequate physical standards and particularly by the use of scientific methods as well as by correlation with other parts of science.

The time seems to be fast approaching when many people will receive formal training in the art of flavor appreciation and control. This art, as it develops, will feed into and draw from all existing pertinent science. The whole art of flavor appreciation is somewhat comparable with the art of musical appreciation. It will be mastered only through long-continued interest combined with and directed by keen sensuous enjoyment. Those fond of living and desirous of the fullest culture will insist on this long study. There can also be short, practical applied courses for dietitians, food processors, students of cooking and of general home economies, and even individuals with a personal flair for this art. Chemists will play an essential part in the development of flavor appreciation, measurement, and control.

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EDITORIAL REVIEW

OBJECTIVE TESTS FOR COOKED FOOD

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One of the cheering signs of the times is the number and kind of objective tests being developed and used for judging or, more properly stated, measuring the properties of cooked food. Subjective tests, with their carefully prepared score cards, have their place and should be used along with the objective, but not as the sole criterion of value since, unlike the objective, they do not provide a permanent record of reproducible results.

A good example of the combined use of both methods of testing is shown by the work done on tenderness and juiciness of meat by various investigators. Lowe (1934) used the dynamometer and penetrometer to measure the tenderness of rare and well-done beef roasts from carcasses graded choice and medium. The results obtained with the dynamometer showed close agreement with the subjective score and the grade of animal; those with the penetrometer were less consistent.

Child and Esteros (1937) used the pressometer to determine the amount of press fluid present in standing and rolled rib roasts of beef. The standing ribs, which yielded the larger amount of juice, were scored higher for quantity, quality, and richness of juice, than were the rolled ones.

Among the objective methods of testing quality of cooked vegetables are those used by Thiessen (1935) who worked on potatoes. She tested tenderness by means of the penetrometer and cellular structure by means of microphotographs and found that the properties thus measured showed considerable variation in potatoes grown on irrigated soil as contrasted with those grown on dry land.

MacGibbon and Halliday (1937) used the Munsell system of notation to study the preservation of green color in the large-quantity cooking of vegetables. Their color measurements in terms of hue, value, and chroma showed clearly the effects of different methods of cooking and holding on the preservation of color.

Numerous devices have been used for measuring the properties of baked products. One of the earliest of these was the shortometer, originated by Davis (1921) and since improved by Dr. C. H. Bailey of Minnesota. This instrument measures "shortness" or tenderness

of crisp products, such as wafers and pastries, by means of the force required to break them; hence it provides a way of determining the relative shortening power of culinary fats, as shown by the work of Fisher (1933) and Noble, *et al.* (1934).

The shortometer obviously cannot be used for cakes; for them Platt and Kratz (1933) devised an instrument which measures tensile strength, through the force required to tear apart a sample of cake of definite size. This instrument, or some modification of it, has been used by a number of workers, among whom is Barmore (1935) of Colorado in his study of angel cakes in the altitude laboratory.

Other properties of cakes that have been measured mechanically are compressibility, one of the factors involved in estimating texture, measured by Platt and Kratz; and relative volume and grain, determined photographically, by numerous workers.

These few illustrations of objective testing suffice to show that we are no longer entirely dependent on the score card for estimating the quality of cooked food. As time goes on, we may hope for an increasing number of workers in the field who are well trained in the physical sciences and capable of devising new tests.

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COMPOSITION OF SUMMER SQUASH AND ITS RELATIONSHIP TO VARIETY, STAGE OF MATURITY, AND USE AS A FOOD PRODUCT

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(Received for publication, May 11, 1937)

INTRODUCTION

It is recorded by Safford (1916) that many kinds of pumpkin and squash *(Cucurbita pepo)* were grown by prehistoric inhabitants of ancient America. Summer squash was cultivated by the Indians at the time early explorers arrived in North America. It was used by early settlers immediately upon their arrival and has remained a popular garden vegetable to the present time.

Summer squash differs from pumpkin and winter squash in that the fruit must be used while still in an immature state. It is consequently important to understand changes in composition as the fruit develops, essential qualities that make it desirable, and characteristics that limit its usefulness as a food product. It is important to know the stage of maturity that makes the most desirable product and to understand the factor or combination of factors which determine this stage. It is of interest to know the extent of the differences in composition of varieties and the degree of suitability of each of these for different methods of preparation and use. The purpose of this report is to present results of some tests bearing upon these points.

Such analyses of summer squash as were available have been averaged by Chatfield and Adams (1931), and the results show that summer squash is composed, on the average, of water, 95 per cent; protein, .6 per cent; fat, .1 per cent; sugar, 1 per cent; starch, .2 per cent; ash, .44 per cent; and fiber, .5 per cent, and that it has a fuel value of 18.9 calories per gram. These results, however, show nothing as to differences in varieties, or effects of stage of maturity or seasonal conditions upon composition. Cordner and Matthews (1931) have studied the composition of White Bush squash at different stages of maturity and they conclude that free reducing substances increase and that the quality of the product improves with age until hardening of the rind makes it inedible.

Summer squash is never used in the raw state; methods of cooking and preparing it for the table are given in most books on cookery.

There seems to be very little reference in the literature to differences in quality of the product at different ages or to differences in varieties.

MATERIALS AND METHODS

The materials for these tests were grown upon a clay loam of moderate fertility on the Arlington Experimental Farm near Rosslyn, Virginia, during the season of 1928. The seed was planted June 7 in rows six feet apart; when the plants were six to eight inches high, they were thinned to two plants per hill, three feet apart. They made only moderate growth at first and on July 5 a top dressing of 300 pounds per acre of nitrate of soda was applied to the soil around the plants. A rain on July 13 made this available to them. They were moderately vigorous and produced an abundant crop of large, well-formed fruits.

Two varieties were grown: a bush scalloped type known as Early White Bush Scallop or White Patty Pan and a yellow crookneck type known as Golden Crookneck. The fruit of the White Patty Pan variety was quite uniform in shape and typical of the variety. The Golden Crookneck was somewhat variable in size and shape, particularly in the length and diameter of the neck. The smoothness or wartiness of the surface was also variable but these variations were no greater than is generally the case with this variety from seed as usually sold.

The plants began setting fruit about June 20, and an ample number of fruits for all samples and tests were tagged with the date when the flowers opened, which permitted harvesting of the fruit at any age desired. Fruits not tagged and not used in the tests were picked as they reached average marketable size.

Fruits for the samples to be analyzed were picked from the vines about 9:30 A.M., brought into the laboratory, and the samples taken as promptly thereafter as possible. A lot of six to 12 fruits of the desired age was used for each sample. A radial section of convenient size was taken from each fruit, chopped finely with a knife, and mixed thoroughly. Duplicate 100-gram samples were weighed out and put into storage containers; 95-per cent alcohol was added to make the concentration 75 to 80 per cent, heated in a water bath to boiling, and the containers were then sealed. Samples were taken at three-day intervals up to 30 days of age with one other sample being taken at 40 days. A separate sample was weighed out for total nitrogen and dried at 75°C. (167°F.).

The alcohol preserved material was extracted as follows: The alcohol was decanted through a weighed filter cup into a separate container, and after grinding the insoluble material with a pestle,

more alcohol was added, which was again decanted through the filter cup. The residual material was then transferred to the cup and washed once or twice with fresh, cold, 95-per cent alcohol. The cup was then placed in a Soxhlet apparatus and further extracted for approximately eight hours with 95-per cent alcohol. In this way the greater part of the soluble constituents was removed before placing in the Soxhlet apparatus, and they were not subjected to the heating that occurs in the final extraction period. After extraction the extracts were combined, made up to volume, and aliquots were taken for the determination of soluble solids, sugars, acids, tannins, and nitrates. The insoluble residue was dried, weighed, and used for the determination of acid hydrolyzable polysaccharides.

The alcohol-soluble solids were determined by drying aliquot portions of the alcoholic extract on an open bath at 60°C.(140°F.) until the alcohol was removed and finally drying completely in a vacuum oven at 75°C.(167°F.). The sugars were determined by the volumetric permanganate modification of the Munson-Walker method as given in the methods of the Association of Official Agricultural Chemists (1920). The titratable acidity was determined by titration with n/10 sodium hydroxide against phenolphthalein as indicator. Total astringency was determined by titration with .05 normal potassium permanganate against indigo carmine as indicator, as specified in the methods of the Official Agricultural Chemists. Total nitrogen was determined by the method recommended by Ranker (1927) for total nitrogen including nitrate nitrogen. Nitrate nitrogen was determined by the ferrous chloride method for nitrates in meats as given in the Official Methods.

Pressure tests were made upon the fruits during the first part of the developmental period, using a pressure tester described by Culpepper and Magoon (1924). Readings were not obtained upon the later stages, as the resistance to pressure was beyond the limits of the instrument.

WEATHER CONDITIONS

The plants developed under weather conditions rather favorable for growth and fruitfulness. A summary is given of the mean temperature and rainfall for June, July, August, and September, which covers the entire period of growth and fruiting of the plants (Table 1). It will be seen that the departures from normal are not very great except as regards the rainfall for August. Rains which totaled 8.6 inches occurred on August 11 and 12. The soil was well drained and consequently the health and vigor of the plants were not impaired. The rains occurred almost in the middle of the sampling period and after the plants were practically fully developed. The rain may have

had some effect upon the composition of later samples although the plants always appeared normal in vigor, and it is believed that the heavy rainfall caused no great departure from the normal course of developmental changes.

TABLE 1

Weather Conditions for the Months of June, July, August, and September, 1928

Month	Mean temperature	Departure from normal	Rainfall	Departure from normal rainfall
	°F.	°F.	*inches*	*inches*
June	71.0	—1.2	2.66	—1.47
July	78.2	+1.4	2.17	—2.54
August	77.0	+2.0	14.41	+10.40
September	64.9	—3.2	4.29	+1.05

ANALYTICAL RESULTS

Solids. Summer squash is characterized by a rather high moisture content, as the percentage of solids or dry matter reached a value as low as six per cent in one of the varieties at one stage of maturity (Table 2). The total solids decreased to the six-day stage and then increased to the 40-day stage in both varieties. The course of the changes is very similar in the two varieties. The solids in the Golden Crookneck are generally somewhat higher than in the White Patty Pan variety. The insoluble solids vary with age and variety in very much the same way as the total solids, decreasing up to six days and then increasing; while the soluble solids vary much less than the insoluble solids, both with age and variety. The soluble solids generally range from four to five per cent, while the values for the insoluble solids may vary from three to eight per cent. In many cases the fruits have almost equal quantities of soluble and insoluble solids. In the younger samples there is a greater proportion of soluble solids, while in the older samples there is a greater proportion of insoluble solids.

Sugars. The total sugar content does not vary widely, the lowest being 2.1 per cent and the highest 3.8 per cent. There is a tendency for the sugar to be minimum in amount in the youngest samples, to increase to the nine-day stage, and then to decrease as the fruits become older. The differences in the varieties are not very great and may not be significant, but there is a tendency for the sugar to be higher in the Golden Crookneck than in the White Patty Pan variety, especially in the more mature fruits. The greater part of the sugar occurs as reducing sugar, the amount of sucrose being less than one per cent in most cases. There appears to be a slightly higher per-

centage of sucrose in the Golden Crookneck than in the White Patty Pan variety at all stages of development. As in the case of the total sugar, the reducing sugar is lowest in the very young and very old fruits and highest in fruits nine to 15 days of age.

Acid Hydrolyzable Polysaccharides. All samples of fruit gave a strong test for starch with iodine, indicating that the greater part of the acid hydrolyzable substances was starch. Like the sugar content, the acid hydrolyzable polysaccharides are not high at any period and are lowest in the early and late stages of development, the highest percentages being found at the 21- to 24-day stage of maturity. It appears, therefore, that the highest content of acid hydrolyzable substances occurs several days after the sugar has reached its maximum and begun to decrease in amount. The acid hydrolyzable substances appear to be somewhat higher in the Golden Crookneck than in the White Patty Pan variety, especially in the later stages of development.

Acidity. The titratable acidity is rather low at all stages of maturity. The percentage present decreases to about the nine-day stage and then again increases somewhat with more mature fruits. The differences seem unimportant, however, from the standpoint of the effect of acidity upon the edible quality of the material. While the acidity appears to be slightly higher in most samples of Golden Crookneck than in those of the White Patty Pan variety of like age, the differences seem unimportant.

Total Astringency. The tannin-like substances in the summer squash appear to be extremely small in amount. An extract of the fruit gave no blue or green coloration with ferric chloride, indicating an absence or extremely low concentration of phenolic substances. The fresh sap that exudes when the fruit is cut, however, has an astringent taste. It is not clear what substances were responsible for the values obtained. The percentage was highest in the fruit at the bloom stage and lowest at the six-day stage, after which there was a tendency to increase somewhat with increase in age, but the differences seem unimportant. Likewise the differences in varieties seem unimportant.

Nitrate Nitrogen. The percentage of nitrate nitrogen is very low in all samples. It increases with age to about the 18-day stage, when it is slightly higher in the White Patty Pan variety than in the Golden Crookneck, then tends to decrease in the older samples, the decrease in the White Patty Pan being much greater than in the other variety. As rainfall totaling 8.67 inches occurred on August 11 and 12, just preceding the taking of the 21-day samples on August 13, the decrease in nitrate nitrogen content might be accounted for by changes in the available supply of nitrates in the soils and may not be due to any

TABLE 2

Composition of Two Varieties of Summer Squash at Different Stages of Maturity[1]

Variety	Date of sampling	Age	Diameter	Alcohol soluble solids	Alcohol insoluble solids	Total solids	Reducing sugars	Non-reducing sugars	Total sugars	Total acid hydrolyzable polysaccharides	Acidity as citric	Total astringency	Nitrate nitrogen	Total nitrogen	Pressure test
		days	*inches*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*gm.*
White Patty Pan.....	July 30	0	1.25-1.5	4.08	3.80	7.88	1.68	0.64	2.32	1.93	.218	.060	.0005	.298	90.8
White Patty Pan.....	July 30	3	2.5 -3.0	3.88	3.09	6.97	2.25	0.41	2.66	1.47	.152	.044	.0008	.208	126.6
White Patty Pan.....	July 30	6	3.5 -4.5	3.80	2.20	6.02	2.06	0.62	2.68	1.00	.150	.040	.0005	.126	162.0
White Patty Pan.....	Aug. 1	9	5.5 -6.0	4.42	2.39	6.81	2.85	0.67	3.52	1.05	.130	.049	.0005	.118	240.5
White Patty Pan.....	Aug. 6	12	5.5 -6.5	4.66	3.17	7.83	2.65	0.58	3.23	1.30	.169	.048	.0009	.119	416.0
White Patty Pan.....	Aug. 9	15	6.0 -6.75	4.66	4.58	9.24	2.98	0.36	3.34	2.00	.175	.056	.0011	.143	510.0
White Patty Pan.....	Aug. 9	18	6.5 -6.75	4.56	5.53	10.09	2.28	0.48	2.76	2.31	.168	.054	.0021	.156	730.0
White Patty Pan.....	Aug. 13	21	6.75-7.5	4.60	5.99	10.59	2.23	0.69	2.92	2.02	.185	.053	.0021	.171	.....
White Patty Pan.....	Aug. 16	24	6.0 -7.5	4.40	6.57	10.97	1.45	1.16	2.61	2.44	.202	.061	.0008	.180	.....
White Patty Pan.....	Aug. 23	27	6.0 -7.5	4.56	6.47	11.03	1.54	0.99	2.53	1.86	.191	.060	.0002	.162	.....
White Patty Pan.....	Aug. 23	30	6.0 -7.5	4.16	5.72	9.88	1.56	0.60	2.16	1.70	.189	.051	.0002	.141	.....
White Patty Pan.....	Sept. 4	40	6.0 -7.5	4.18	6.35	11.53	1.66	0.67	2.33	1.78	.181	.060	.0002	.122	.....
Golden Crookneck.....	July 27	1	1.0 -1.25	4.28	4.32	8.60	1.06	1.10	2.16	1.60	.254	.126	.0004	.366	97.0
Golden Crookneck.....	July 28	3	1.25-1.75	4.16	3.90	8.06	1.92	0.86	2.78	2.37	.146	.063	.0005	.310	119.3
Golden Crookneck.....	July 28	6	1.5 -2.25	4.06	3.89	7.95	2.19	0.83	3.02	2.51	.140	.041	.0010	.260	160.0
Golden Crookneck.....	Aug. 1	9	3.0 -3.5	4.24	4.05	8.29	2.83	0.95	3.78	2.40	.130	.043	.0011	.206	191.2
Golden Crookneck.....	Aug. 6	12	3.0 -3.75	5.22	5.02	10.24	2.54	1.08	3.62	2.86	.185	.045	.0013	.244	342.5
Golden Crookneck.....	Aug. 9	15	3.5 -4.5	5.12	5.88	11.00	2.13	1.55	3.68	3.59	.217	.076	.0018	.251	529.6
Golden Crookneck.....	Aug. 8	18	3.5 -4.75	4.58	5.71	10.29	1.70	1.78	3.48	3.67	.207	.069	.0019	.239	.....
Golden Crookneck.....	Aug. 13	21	3.5 -4.75	4.54	6.84	11.38	2.11	1.41	3.52	3.83	.211	.061	.0009	.284	.....
Golden Crookneck.....	Aug. 16	24	3.5 -4.75	4.66	6.70	11.36	2.12	1.50	3.62	3.34	.214	.077	.0013	.217	.....
Golden Crookneck.....	Aug. 16	27	3.5 -4.75	4.58	7.47	12.05	1.46	1.48	2.94	2.92	.224	.076	.0012	.233	.....
Golden Crookneck.....	Aug. 24	30	3.5 -4.75	4.30	8.30	12.60	2.03	0.59	2.62	2.90	.219	.074	.0006	.254	.....
Golden Crookneck.....	Sept. 2	40	3.5 -4.75	4.64	8.18	12.82	1.94	0.76	2.70	2.59	.221	.091	.0008	.217	.....

[1] Expressed as percentage of the fresh weight.

influence of age of the material. However, it is probable that both the age of material and the supply available in soil are important factors influencing the nitrate content of the fruit. The differences in varieties are very small and do not appear to be important.

Total Nitrogen. The total nitrogen is highest in the fruit at the bloom stage and decreases to about the nine-day stage, after which the changes are rather irregular but exhibit a tendency to increase up to 21 or 24 days then decrease again in the very old material. It is evident that on the fresh-weight basis the protein of summer squash is low; however, it makes up a substantial portion of the dry matter of the material, nearly 25 per cent in the very young fruit and from 10 to 15 per cent in fruits nine to 15 days of age. The Golden Crookneck was slightly though consistently higher in total nitrogen at all stages than was the White Patty Pan variety.

Pressure Tests. It may be noted that in contrast to the small changes in chemical composition the pressure tests indicate a very striking change in physical character of the fruits with increase in age. There is a continual increase in resistance to puncture with increase in age, being moderately rapid to about the nine-day stage when the rate of increase becomes more marked, continuing until the resistance to pressure reaches the limits at which it can be measured by the instrument employed.

CANNING TESTS

There is very little mention of the use of summer squash for canning purposes. Breazeale (1909) describes methods of canning it in the home. From the growth rates and the developmental changes it obviously might be advantageous to preserve the material in some manner when in its optimum condition for use. Accordingly, a few tests were made in order to note the differences in varieties and the effect that stage of maturity may have upon quality of the product.

Material for the tests was picked at approximately 9:30 A.M., brought into the laboratory, washed and peeled by hand, removing a thin surface layer with as little of the underlying tissues as possible. The fruits were then cut into cubical pieces one to two inches in size or thickness, steamed for 30 minutes in flowing steam, passed through a food chopper, and the pulp filled into No. 2 tin cans while still hot. The cans were sealed immediately, processed one hour at 15 pounds pressure, and promptly cooled in running water. They were thus handled by a method very similar to that employed for canning pumpkin. Representative lots of material were canned at six, nine, and 12 days of age. A part of each of the lots was canned in plain tin and in enameled cans such as are generally used for canning

[1] Expressed as percentage of the fresh weight.

pumpkin. At the end of five months cans from each of the lots were opened and the texture, flavor, and color compared. Consistency tests were made by means of a consistometer originally devised for measuring the consistency of greases and petrolatum in accordance with specifications of the American Society for Testing Materials for test D-217-27T (1927). The cone and attachments weighed 150 grams. The cone was released for five seconds and the distance it penetrated in each case was measured and the results expressed in millimeters.

In comparing quality of the product, the judgment and preferences of a number of individuals were relied upon. The ratings were made upon a numerical basis, the scale ranging from 1 to 10. The product of lowest quality or the one that was least desirable might be given a rating of 10, while products of higher quality were given

TABLE 3

Results of Canning Tests Showing Effect of Variety and Stage of Maturity on Color, Texture, and Flavor of Summer Squash

Variety	Age	Diameter	Pressure test before canning	Consistency after canning	Numerical rating[1]			
					Color	Flavor	Texture	General preference
	days	*inches*	*gm.*	*mm.*				
White Patty Pan...	6	3.5 -4.5	162.0	38.9	7.0	4.0	1.5	3
White Patty Pan...	9	4.5 -6.0	240.5	38.1	6.0	6.0	2.5	5
White Patty Pan...	12	5.0 -6.5	416.0	37.3	5.5	6.0	6.0	6
Golden Crookneck..	6	1.5 -2.5	160.0	37.5	5.0	1.0	1.0	1
Golden Crookneck..	9	3.0 -3.5	191.0	36.5	3.0	2.5	1.5	2
Golden Crookneck..	12	3.25-3.75	342.5	35.0	1.0	3.0	3.0	4

[1] Values given for these factors are based on personal preferences of a number of individuals upon tasting and examining the product.

lower numerical ratings in proportion to their excellence, with a rating of 1 being the highest quality. A rating of 10 would indicate that the product was decidedly unsuitable for table use (Table 3).

It may be noted that the canned product was in all cases a semi-solid of attractive appearance and characterized by a low acidity and mild but pleasing flavor. The product showed a pronounced tendency to flatten out or flow to some extent over the surface of the dish when it was emptied from the can. Also a small amount of liquid tended to separate from the solid portions on standing.

Consistency. The consistency of the product did not vary greatly at any of the stages here canned. In both varieties the readings for consistency are somewhat higher at the six- than at the 12-day stage of maturity, indicating a sinking of the cone to a greater depth and consequently a thinner product in the younger material. It is to be noted that this is what would be expected from the analytical results,

since both the total solids and the acid hydrolyzable polysaccharides are lower at the six-day stage than at the 12-day stage. It is evident that the starch content is too low to prevent the separation of some liquid on standing.

The White Patty Pan variety made a product which was somewhat thinner than the Golden Crookneck, probably in consequence of the somewhat lower solids and starch content of the variety. It should be remembered that these differences are small and it may be expected that variations in climatic conditions or sampling errors may account for variations of this extent.

Color. In the processing a certain amount of browning occurred as a result of the high temperature employed, but this appeared less marked than might have been expected. The low acidity minimizes the effect of the high temperature in the processing. The chemical analyses show that the tannin and related substances are very low. The practical absence of tannin or the small amount present, probably accounts largely for the small amount of discoloration which occurred. In the case of the White Patty Pan variety age of the material did not greatly affect color of the product. The older material appeared a little lighter and was slightly preferred by most of the judges. In the Golden Crookneck the color steadily improved with age. This variety contains a yellow pigment which is insoluble in water and soluble in the fat solvents, undoubtedly a carotinoid pigment. All the judges preferred a product with a deep yellow color, and the Golden Crookneck was therefore rated much higher on color than the White Patty Pan.

Flavor. Summer squash has a flavor somewhat like that of winter squash but milder or less intense. The high temperature employed in processing the material altered its flavor somewhat but it still possessed its natural flavor to a marked degree. Flavor did not vary greatly with the age of samples here tested but most of the judges preferred to some degree the younger material. However, there were some differences of opinion. All the judges preferred the flavor of the Golden Crookneck variety to that of the White Patty Pan, rating it several points higher.

Texture. The texture of the product varied considerably with age, the younger material being much preferred by all the judges. The young material was tenderer, finer grained, and less fibrous than the older material. There was not a great difference between the varieties except at the 12-day stage, in which case the White Patty Pan was much less desirable because of its coarseness and the presence of the developing seed. In the young fruits the seeds are of no significance but as the fruits attain greater age the seeds become noticeable and

finally become so prominent as to make the material unpalatable. However, in the three stages here canned the fruits made a fairly acceptable product in each case, the 12-day stage being considered the upper limit at which most individuals would use the material.

General Preference. The product most desirable when all factors were considered was judged to be the six-day stage of the Golden Crookneck variety. The young material was preferred in both varieties, to some extent by reason of its more pleasing flavor but very largely because of its finer grained, more desirable texture. The Golden Crookneck was preferred principally because of its color but also to some extent because of its better flavor and texture, especially in the older material.

Corrosion of the Can. Summer squash behaves somewhat like pumpkin in its effect upon a tin can. In a plain tin can at the end of five months there was no serious corrosion, but there were numerous darkened areas distributed irregularly over the inner surface. A slight etching was also evident which became considerably more pronounced at the end of 15 months, when the entire lots of cans were opened to make the observations. Culpepper and Moon (1928) have noted that small amounts of nitrates greatly accelerate the process of corrosion in the tin can. It is suspected that the presence of nitrates is largely responsible for corrosion in this case. They occur in small amounts in all samples here analyzed. Since it is known that the nitrate content of the plant is greatly influenced by the amount present in the soil, it might be expected that under different conditions the corrosive action of summer squash would be much greater or much less than in this case, depending upon the supply available in the soil. Very little action on the enameled can could be observed and it was evident that the enamel satisfactorily protected the metals of the can.

COOKING TESTS

In order to supplement and extend observations on quality of the material at different stages of maturity a number of simple cooking tests were carried out. Preparation of the squash was exactly the same as for the canning tests. After being peeled and sliced, the material was steamed for 30 minutes, as described in the canning tests. This appeared to be somewhat longer than was necessary to cook the material, for a few tests indicated that 20 minutes was generally sufficient, but the additional cooking seemed to make little difference in quality. After steaming, salt was added and the material was thoroughly mixed and mashed, as is generally done in preparing it for the table. Tests were made on samples differing by three-day intervals from three to 21 days of age. The ratings given are based

almost entirely upon preferences of the author and members of the laboratory where the work was done. In some older samples the outer layer, which had hardened considerably, was removed by somewhat deeper peeling. The seed or seed cavity was not removed except in special tests where the wall of the fruit alone was used. The system of rating the cooked product was similar to that used for the canned product (Table 4).

TABLE 4

Results of Cooking Tests Showing Effect of Variety and Stage of Maturity on Color, Flavor, and Texture of Summer Squash

Variety	Age	Diameter	Pressure test before cooking	Numerical rating[1]			
				Color	Flavor	Texture	General preference
	days	*inches*	*gm.*				
White Patty Pan	3	2.0 -3.0	126.6	8.0	2.5	1.0	2.0
White Patty Pan	6	3.5 -4.5	162.0	6.0	2.5	1.5	2.5
White Patty Pan	9	4.5 -6.0	240.5	6.0	3.5	2.0	3.0
White Patty Pan	12	5.5 -6.5	416.0	6.0	4.0	4.0	5.0
White Patty Pan	15	5.5 -6.75	510.0	6.0	4.0	6.0	6.0
White Patty Pan	18	5.5 -7.0	730.0	7.0	4.0	10.0	10.0
White Patty Pan	21	5.5 -7.0	.....	7.0	4.5	10.0	10.0
Golden Crookneck	3	1.25-1.75	119.3	3.0	1.5	1.0	1.0
Golden Crookneck	6	1.5 -2.5	160.0	2.5	1.0	1.5	1.5
Golden Crookneck	9	3.0 -3.5	191.2	2.5	1.0	1.5	2.0
Golden Crookneck	12	3.0 -3.75	342.5	1.5	1.5	2.0	2.0
Golden Crookneck	15	3.0 -4.5	529.6	1.0	2.5	4.0	3.0
Golden Crookneck	18	3.5 -4.75	.....	1.5	3.0	7.0	6.0
Golden Crookneck	21	3.0 -4.75	.....	1.0	3.0	8.0	9.0

[1] Values given are based upon personal preferences of the judges.

Stage of Maturity. As in the case of the canned product, the young material was preferred in both varieties. Even the three-day stage was preferred slightly to the six-day stage because it seemed to be a little richer to the taste and was finer grained and smoother in texture. It appears that texture is the most variable and the most important character determining quality and usefulness of the material as a food product. Next in importance is color of the product, but the color is never such as to make the product unsuitable for table use. A deep yellow color is generally preferred, but it is by no means essential. Summer squash at all stages of maturity is more nearly free from dark discolorations that occur on exposure to the air than is the case with many other fruits and vegetables. As was stated in the discussion of the canned product, the freedom from discoloration is probably due to the absence or low content of tannin and related substances. The variations in sugar, acid, and solids content at different stages of maturity are not great enough to be very important

in determining quality of the product. The flavor, while somewhat variable, was in no case such as to preclude the use of the material for the table. The results indicate that quality of the product from the consumer's standpoint slowly but steadily declines to the 12- or 15-day stage and then quickly becomes unpalatable, apparently because of increasing prominence of the developing seeds and changes in the coarseness and fiber content of the tissues.

Varieties. The Golden Crookneck has been generally preferred to the White Patty Pan, very largely because of its color. The solids, sugars, starches, and protein appear to be slightly higher in the Golden Crookneck variety, but the differences are too small to be of great importance. There is considerable difference in the texture of the two varieties, especially in the later stages of development. The White Patty Pan variety has a very thin wall, the greater part of the fruit consisting of the seed cavity. When this portion becomes fibrous and the seeds become large enough to be objectionable, the whole fruit becomes undesirable as a table product. In the case of the Golden Crookneck the wall is thicker, the seed cavity is smaller, and the neck is entirely solid, being composed of wall tissue which results in the seeds and fibers of the seed cavity being proportionately less in amount. It is thus possible to use the Golden Crookneck at a somewhat older stage of maturity than the White Patty Pan variety. Golden Crookneck is much more nearly like winter squash. After it is too old to be used in its entirety, the seeds and fibers surrounding them may be removed, and by careful peeling a large portion of the fruit is left which still makes a very acceptable table product. It may be used in this way even up to the 18- or 24-day stage of maturity.

Pressure Test Before Cooking. It may be noted that texture of the product is very closely correlated with resistance to pressure of the surface of the fruit before cooking. The texture of the cooked entire fruit depends upon development of the seeds and fiber of the tissues, while values for the pressure test are determined by hardening of the rind or outer layers. Development of the seed and hardening of the rind sufficiently to be objectionable occur at nearly the same time, but these results indicate that the seeds and fibers begin to alter considerably the quality of the product a short time before the rind becomes hard enough to be objectionable. When the fruits gave a pressure test of 500 grams, the rind had hardened sufficiently to be somewhat objectionable.

Time of Harvest. It is evident from the above results that the proper stage of maturity at which to harvest the material must be a compromise between yield and convenience of harvest, on the one hand, and quality of the product, on the other. Judging from size of

fruits at different stages the yield would increase at least up to the 12- to 15-day stage of maturity, while the quality decreases from five or six days onward. Material three days old is considered too small to be economically harvested and prepared for the table. The most favorable stage for harvest, all things considered, is six to nine days of age for material harvested in the middle of summer. This appears to be very close to the stage of harvest in good practice at the present time. It is evident that the fruit should be harvested before it has reached full size. It has been observed by a number of investigators and growers that yield per plant may be actually increased by harvesting when the fruits are rather young, since the plants then continue to grow and set fruit throughout the summer, whereas they cease to grow or to set fruit abundantly if the fruits are allowed to remain on the vine until towards the end of the stage at which they may be used for the table.

Discussion. It should be noted that composition as well as quality of the product will undoubtedly vary somewhat with climatic and soil conditions. Also, preferences of consumers may vary in different sections of the country, as well as from time to time. Therefore, it cannot be expected that the composition and physical characteristics of the fruit used in these tests would be exactly the same under all conditions or that the ratings of the products would apply exactly in all cases, but this should in no way invalidate the conclusions as to the relationship between the physical and chemical characteristics of the material and its quality as a table product. Also, it should be remembered that the rate of development depends largely on the prevailing temperature and hence would be slower later in the season or in cooler or more northern sections of the country.

SUMMARY

A study of the composition and quality as a food product was made of two varieties of summer squash at several stages of growth and maturity.

The dry matter or the total-solids content of summer squash was rather low as compared with many other vegetables. Total solids decreased somewhat to the six-day stage and then increased to complete maturity.

The total sugar did not vary greatly but increased slowly to the 15-day stage and then decreased to the 40-day stage.

The acid hydrolyzable polysaccharides tended to increase to the 18- or 24-day stage and then decrease somewhat to the 40-day stage.

The titratable acidity and the tannin-like substances were very low in amount and did not vary widely at any stage of maturity.

The total nitrogen or the protein content decreased to the nine-day stage after which the variations did not appear very significant but exhibited a tendency to increase for a short time and again decrease in the very old stages.

There was a small amount of nitrate nitrogen present at all stages of maturity. It increased to about the 18-day stage and then decreased in older material. There is a tendency for the Golden Crookneck to be slightly lower in moisture and slightly higher in nearly all other constituents than is the White Patty Pan variety.

Cooking and canning tests made at stages of maturity corresponding to ages of the samples analyzed indicate that the solids, sugars, acids, tannins, and proteins do not vary widely enough to alter greatly the value of the product as a food material. The quality of the product is influenced to some extent, however, by such variations as occur.

The texture of summer squash appears to be the factor that is most important in determining its quality and palatability. A deep yellow color appears to be desirable but is not essential to the palatability or appearance of the product. A low tannin content makes the product rather free from dark discolorations that might otherwise occur.

In these tests the quality was judged to be highest in young material. When all factors are considered, however, the most favorable period of harvest appears to be between the six- and the nine-day stage of maturity for summer squash developing in the middle of summer in regions having the same summer temperature as does Washington, D. C.

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STUDIES ON PHENOTHIAZINE
I. A COLORIMETRIC METHOD FOR ESTIMATION OF PHENOTHIAZINE[1]

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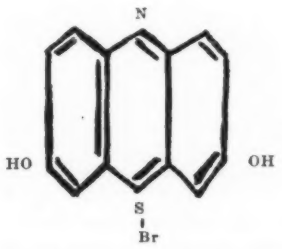
(Received for publication, May 18, 1937)

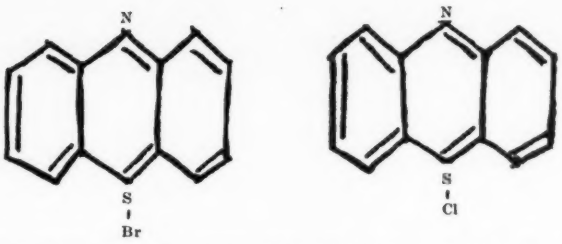
When hydrogen peroxide is added to an acid alcoholic solution of phenothiazine, heated to about 80°C.(176°F.), the phenothiazine is converted to a highly colored compound which we believe to be 9-hydroxyphenthiazone, also known as thionol. A detailed description of this method will be given in a later paper. The quantitative conversion of known amounts of phenothiazine to thionol, however, requires a fairly close adjustment of the amounts of acid, alcohol, and hydrogen peroxide relative to the quantity of phenothiazine. Therefore, this method of producing the highly colored thionol from an unknown quantity of phenothiazine cannot conveniently be made the basis of an accurate colorimetric method for estimating phenothiazine.

Fortunately, however, when phenothiazine is treated with saturated bromine water, under appropriate conditions, a red alcohol-soluble compound is formed. This compound is believed to be 3, 9-dihydroxyphenazothionium bromide, or orthoquinoid thionol hydrobromide with the following formula:



Support for this belief is found in the following quotation from Von Richter (1923): "By oxidation with $FeCl_3$, or bromine, thiodiphenylamine and its homologues, like phenoxazine, are converted into coloured ortho-quinoid so-called 'azo-thionium' salts (with quadrivalent

[1] Food Research Division Contribution No. 330.



sulphur), which like the azoxonium salts are substituted, on treatment with amines or water, in the para-position, with respect to the N-atom, by NHR or OH groups."

The depth of color so produced by conversion of a known amount of phenothiazine is reproducible, and within certain limits the depth of color is a straight line function of the quantity of phenothiazine when examined in a photoelectric colorimeter, described by Eddy and DeEds (1937), (Fig. 1). These facts have made possible the

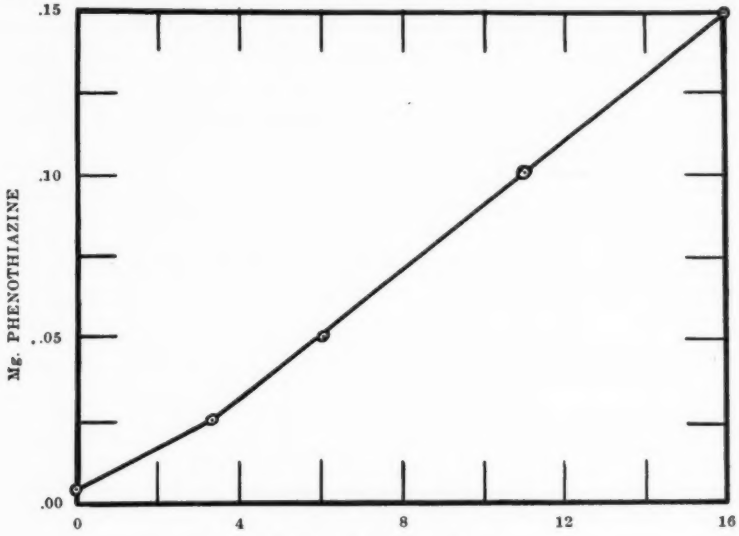


FIG. 1. Colorimeter calibration curve for phenothiazine.

development of a colorimetric method for the estimation of phenothiazine. The method is applicable to the determination of phenothiazine spray-residues on apples and has been used in this laboratory for that purpose to obtain data which would aid in the interpretation of studies on chronic toxicity of phenothiazine.

EXPERIMENTAL PROCEDURE

In the procedure for the colorimetric determination of phenothiazine the following reagents are necessary: 95 per cent ethyl alcohol, saturated bromine water, phosgene-free chloroform, and .1 per cent alcoholic phenothiazine solution. This stock solution of phenothiazine was diluted as required to give a standard containing .05 mg. phenothiazine per c.c.

The standards were prepared by oxidizing known quantities of phenothiazine. To six Erlenmeyer flasks of 50 c.c. capacity, designated A, B, C, D, E, and F, were added .10, .50, 1.00, 1.50, 2.00, and 2.50 c.c., respectively, of the diluted stock solution. Alcohol was added to bring the total volume to 10 c.c. Two c.c. of the saturated bromine water were added and the solution allowed to stand for five minutes. The flasks were then placed on a hot plate and warmed for

TABLE 1

Efficiency of Phenothiazine Solvents

Apple No.	Phenothiazine added	Phenothiazine found	Recovery	Remarks
	mg.	*mg.*	*pct.*	
B 17	1.00	.30	33.0	Carbon tetrachloride wash. Wax not removed.
B 20	0.50	.40	80.0	Benzene wash. Wax not removed.
B 14	0.50	.425	85.0	Chloroform wash. Wax not removed.
B 22	1.00	.985	98.5	Alcohol wash. Wax not removed.
B 22-2	.....	.005	0.5	Second alcohol wash. Wax not removed.
B 22-3	.....	.001	0.1	Third alcohol wash. Wax not removed.
B 9	1.50	.75	50.0	Alcohol wash. Wax removed by filtration.
B 10	1.20	.45	37.5	Alcohol wash. Wax removed by filtration.

five minutes, just to the boiling point. After the flasks were cooled to room temperature, 10 c.c. of chloroform were added and the total volume made up to 25 c.c. with alcohol. Owing to a slight change in the color when chloroform was added, it was necessary to allow 10 minutes to elapse before using the standards for comparison. The comparisons may be made in Nessler tubes or in the photoelectric colorimeter described by Eddy and DeEds.

In order to apply this method to the determination of phenothiazine in spray-residues on apples, it was necessary to consider the following points: (1) the most satisfactory solvent for removal of phenothiazine, (2) volume of and method of applying this solvent to remove the phenothiazine, and (3) effect of apple-wax removed on recovery of phenothiazine. Representative data concerning these factors are given (Table 1).

The data on Apples B 17, B 20, B 14, and B 22 show that when apple-wax is not removed by filtration, the order of increasing efficiency for solvents for phenothiazine is carbon tetrachloride, benzene, chloroform, and alcohol. When alcohol is used as a solvent, removing the apple-wax by filtration markedly reduces the amount of phenothiazine recovered, as shown by the results on Apples B 9 and B 10. This suggests that some of the phenothiazine is adsorbed on, or dissolved in, the apple-wax. The data on Apples B 22-2 and B 22-3, compared with B 22, show that a second washing with alcohol suffices. The presence of apple-wax had no effect on the accuracy of analysis if the clouding produced in the alcoholic washings by the wax was cleared by the addition of chloroform to the red 3, 9-dihydroxyphenazothionium bromide solution. It was also shown that ursolic acid, at least in quantities up to .025 gm. in 100 c.c. of alcohol wash, had no effect on the recovery of phenothiazine from control apples.

The following method was used to determine phenothiazine on apples which had received two sprays of lead arsenate and seven cover sprays of phenothiazine. The apple was placed in a beaker of 600 c.c. capacity and 50 c.c. of boiling alcohol were poured over it, the skin being scrubbed with a small, stiff test-tube brush to aid in the removal of phenothiazine spray particularly around the stem and blossom ends. The alcoholic washing was poured off and the process repeated with a second 50-c.c. portion of boiling alcohol. The apple was then removed from the beaker and rinsed with about 10 c.c. of alcohol. The combined alcoholic washings were evaporated on a steam bath to a volume such that the amount of phenothiazine in 10-c.c. aliquot portions lay within the range selected in the standards. Ten-c.c. aliquot portions were then used for development of the red 3, 9-dihydroxyphenazothionium bromide, using the same procedure employed in preparing the standards.

The applicability of this method to apples was shown by analyses made on apples not subjected to phenothiazine sprays (Group C), apples to which known amounts of phenothiazine had been added in the laboratory (Group B), and apples which had received two sprays of lead arsenate and seven cover sprays of phenothiazine (Group A). The results are summarized (Table 2).

The data show that (1) apples which had never been subjected to phenothiazine sprays consistently gave blank values corresponding to less than .005 mg. phenothiazine; (2) known amounts of phenothiazine added to apples, by evaporating an alcoholic solution of phenothiazine from the surface of the apple, can be quantitatively recovered; and (3) the phenothiazine content of a series of apples

receiving seven cover sprays of phenothiazine varies from about 4.5 to 8.2 γ phenothiazine for each square centimeter of apple surface (Table 2).

TABLE 2

Phenothiazine Recovery

Apple No.	Area of apple	Phenothiazine added	Phenothiazine found	Recovery	γ per sq. cm.
	sq. cm.	*mg.*	*mg.*	*pct.*	
B 23	.....	0.500	0.525	105	.....
B 26	.....	1.000	1.000	100	.....
B 27	.....	1.000	1.000	100	.....
B 28	.....	0.500	0.500	100	.....
B 29	.....	0.500	0.500	100	.....
C 2	.....	0.000	<0.005	.....	.....
C 3	.....	0.000	<0.005	.....	.....
C 4	.....	0.000	<0.005	.....	.....
C 5	.....	0.000	<0.005	.....	.....
C 6	.....	0.000	<0.005	.....	.....
A 11	186.2	.....	0.835	.....	4.5
A 12	176.6	.....	1.300	.....	7.4
A 13	145.2	.....	1.185	.....	8.2
A 14	176.6	.....	0.850	.....	4.8
A 15	153.9	.....	0.775	.....	5.0

SUMMARY

The conversion of phenothiazine to a highly colored red compound, believed to be 3, 9-dihydroxyphenazothionium bromide, has been made the basis of a colorimetric method for the quantitative estimation of phenothiazine. The applicability of the method to the estimation of phenothiazine in apple-spray residues has been demonstrated.

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VITAMIN STUDIES ON BANANAS
I. THE VITAMIN A, B_1, and C CONTENTS OF RIPE BANANAS

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Since the inception of the vitamin theory, bananas have usually been considered a good source of such essential food factors. Textbooks list the potency of bananas as + to ++ for vitamin A, + to ++ for vitamin B (B_1), ++ for vitamin C, and ++ for vitamin G (B_2). One + indicates that the food contains the vitamin, while ++ indicates that the food is a good source of the vitamin. The vitamin D potency of bananas, in common with other fruits and vegetables, is low.

The establishment of minimum vitamin requirements for children and adults by Rose (1933), Steibling (1936), and others and the use of International Reference Standards in bioassay techniques have brought about the necessity for a re-analysis of our common foodstuffs for their vitamin content. The present study contains the results of vitamin A, B_1, and C assays carried out by latest approved techniques upon ripe banana pulp. The bananas used were representative of the banana of commerce in the United States.

VITAMIN A

The method used to measure the vitamin A content of bananas was that adopted as official by the U. S. Pharmacopoeia (U.S.P.X., Interim Revision Announcement No. 2, 1934). In addition to the U.S.P. reference cod liver oil which contained 3,000 International vitamin A units per gram, pure beta-carotene (S.M.A.) was used as a reference standard. The data obtained in this assay are shown (Table 1) and results given (Figs. 1, 2, and 3).

In accordance with the U.S.P. method of calculating vitamin A potency, 300 mg. of banana pulp, which induced a growth response equal to .333 mg. of the reference cod liver oil (one I. U.) and greater than .6 gamma of beta-carotene (one I. U.) are considered to contain one International vitamin A unit. As commonly expressed, this is equivalent to 95 International vitamin A units per ounce of banana. Since the chemical constituents of natural foodstuffs vary in amount owing to a multitude of conditions, such as soil composition, time of harvesting, percentage of moisture present, etc., it was considered advisable to express vitamin unitage as a range rather than as a

single value. Consequently, 95 units per ounce may conservatively be called the maximum value and 71 units per ounce may be considered minimum, since 400 mg. banana obviously (Table 1) contain more than one International unit.

A second method for calculating the vitamin potency was also used. The unit potency of 300, 400, 500, and 600 mg. was found by inspection (Fig. 2). This potency was found to be 1.0, 1.1, 1.1, and 1.45 I. U., respectively. These values give an average figure of 77 ± 5 I. U. per ounce of banana. This falls within the limits of 71 to 95 I. U.

TABLE 1

Average Weight Gains of Rats Used in the Vitamin A Assay of Ripe Banana Pulp

Supplement	Amount supplement fed daily	Number of animals	Av. wt. gain in 28 days	Vitamin A potency
	mg.		*gm.*	*I. U. per oz.*
Cod liver oil (U.S.P.)	0.333	6	15	(85,050)
Cod liver oil (U.S.P.)	0.500	7	39	(85,050)
Cod liver oil (U.S.P.)	1.000	7	48	(85,050)
Beta-carotene	0.0006	7	13	(47,250,000)
Control group	....	10	All died.	....
Banana	200.0	4	6	....
Banana	300.0	6	15	95
Banana	400.0	12	22	71
Banana	500.0	12	21	....
Banana	600.0	9	36	....
Banana	1000.0	2	43	....

obtained by the approved method of calculation. The value used by Sherman (1936) as the average vitamin A potency of ripe banana pulp is 67 ± 8 Sherman units per ounce. Using the usual conversion factor (1.4) this value is approximately 94 ± 11 International units per ounce, which is slightly higher than the value found in this laboratory.

The similarity of the average weight gains made by the group fed 400 mg. and 500 mg. of banana (Table 1) presented an apparent anomaly. However, an examination of the curves for these two groups (Fig. 1) afforded a probable explanation. The animals which received 500 mg. of banana were depleted of their stored vitamin A so completely that for the first week upon experiment they continued to lose weight. From that time, however, their weight gain was greater and more rapid than the group which received 400 mg. of banana.

VITAMIN B_1

The technique used in the assay of bananas for vitamin B_1 content was that proposed in 1934 by the American Drug Manufacturers Asso-

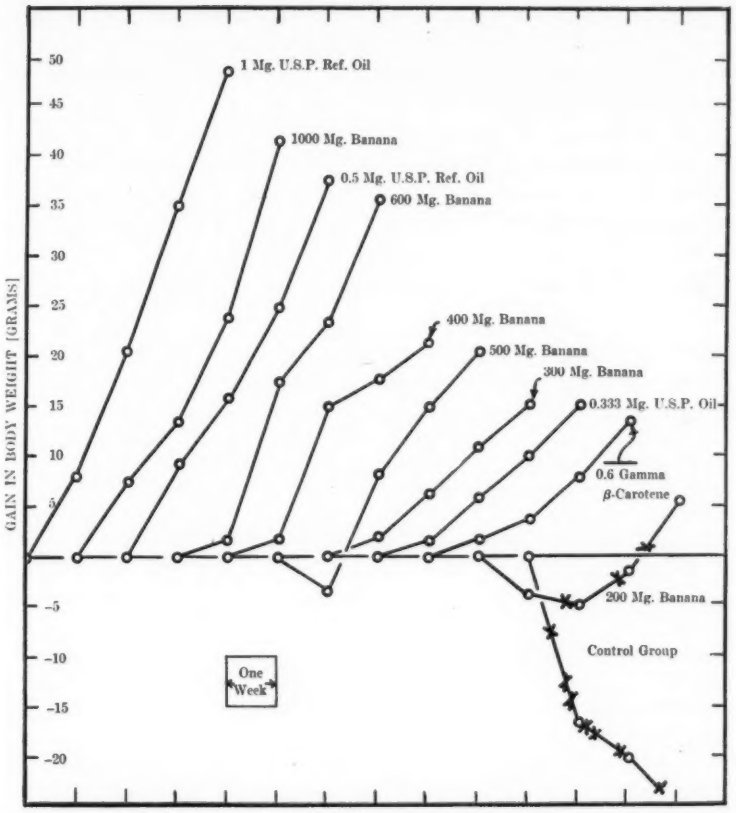

FIG. 1. Effect on body weight of supplement fed in vitamin A assay.

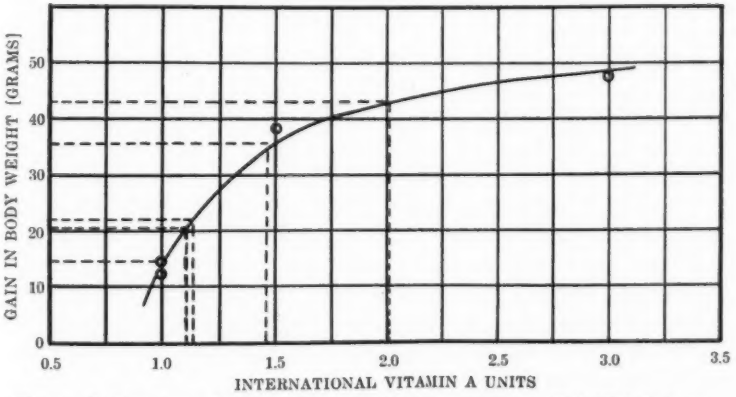

FIG. 2. Laboratory reference curve for vitamin A assays. (Established from U.S.P. reference cod liver oil and beta-carotene.)

ciation for official adoption by the U. S. Pharmacopoeia. The International vitamin B_1 adsorbate and crystalline vitamin B_1 (Merck) were used as reference standards. Data obtained in this assay are shown (Table 2 and Fig. 4).

By definition, 10 mg. of the International vitamin B_1 standard contain one International vitamin B_1 unit. It is also fairly well established by Waterman and Ammerman (1935) that five gamma of crystalline vitamin B_1 is equivalent to one I. U. Therefore, since six grams of ripe banana pulp induced a biological response better than either of the standard reference materials, this quantity of banana

TABLE 2

Summary of Results of the Vitamin B_1 Assay of Bananas

Supplement	Amount supplement fed daily	Number of animals	Av. wt. gain in 28 days	Potency
			gm.	*I. U. per oz.*
International vitamin B_1 standard adsorbate	10 mg.	9	24	(2,835)
Crystalline vitamin B_1	5 gamma	12	22	(5,670,000)
Control group	....	8	All died of typical vitamin B_1 deficiency.	....
Banana	4 gm.	3	4	....
Banana	6 gm.	10	25	4.7
Banana	7 gm.	8	27	....
Banana	8 gm.	2	36	....
Banana	10 gm.	3	46	....

must contain one I. U. In a more commonly stated form this is equivalent to 4.7 I. U. per ounce. In accordance with the prevailing tendency to express vitamin potency of foodstuffs as a range, the vitamin B_1 value of ripe banana pulp may be considered as four to five I. U. per ounce. This value is somewhat lower than that found by Baker and Wright (1935). In a study of English foods, using as their method the measurement of progressive bradycardia in rats, they attribute to ripe bananas a value of 14 to 15 I. U. per ounce. An explanation for the difference between their results and ours probably lies in the difference in variety of fruit assayed. A part of the bananas imported by England are of the *Musa Cavendishii* variety, a slightly smaller banana than the *Musa sapientum* which is the banana of commerce in the United States. Since the International vitamin B_1 adsorbate was also used by Baker and Wright as a reference standard, their results and ours should be approximately equal for the same kind of fruit regardless of the technique employed.

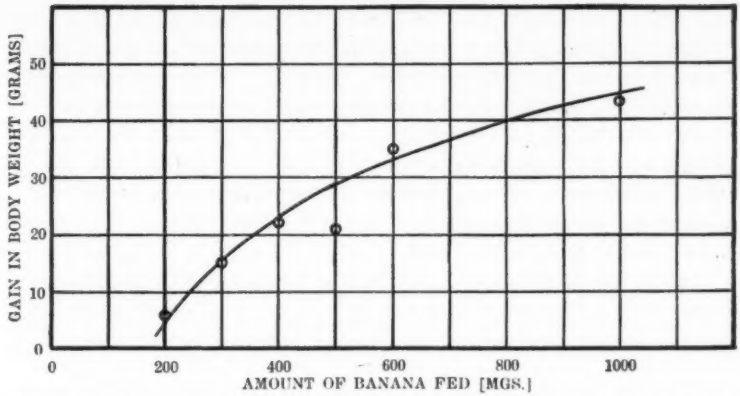

FIG. 3. Growth response induced by vitamin A in various amounts of banana pulp.

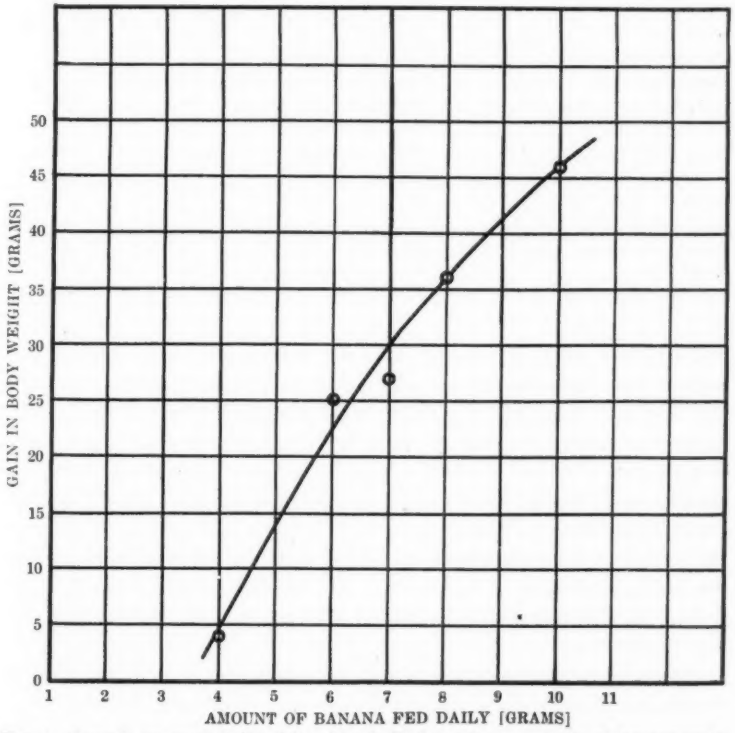

FIG. 4. Growth response induced by vitamin B_1 in various amounts of banana pulp.

VITAMIN C

Several investigators in the past two decades have assayed bananas for vitamin C with results as shown (Table 3). Some of these workers reported their results as the minimum quantity, in grams of banana, required to protect guinea pigs from scurvy symptoms. This minimum protective dose is considered to contain .5 mg. of ascorbic acid. Therefore, for purposes of easy comparison, the values in the table are expressed as milligrams of ascorbic acid per gram of banana pulp and as International units per ounce.

It was felt that the range of 17 to 113 International units expressed a variation in vitamin C potency of bananas somewhat greater than

TABLE 3

Vitamin C Content of Bananas as Determined by Various Investigators

Authority	Method	Ascorbic acid	International vitamin C units
		mg. per gm.	*I. U. per oz.*
Lewis (1919)	Biological response	.03 to .05	17 to 28
Givens, *et al.* (1921)	Biological response	.05	28
Jansen and Donath (1925)	Biological response	.05 to .10	28 to 57
Eddy and Kellogg (1927)	Biological response	.10	57
Grunninger (1928)	Biological response	.05	28
Eddy (1929)	Hojer technique	.05	28
Naeslund (1931)	Biological response	.05	28
Dalldorf (1931)	Capillary resistance	.05	28
Birch, *et al.* (1933)	Chemical titration	.15	85
Davies and Ziegler (1936)	Chemical titration	.042	25
Eddy (1936)	Chemical titration	.20	113
Leverton (1937)	Chemical titration	.073	41

that which is actually true. Therefore, it became the purpose of this part of the study to (1) determine the minimum protective dose of ripe banana pulp and (2) to correlate chemical titration values for vitamin C with the bioassay results. This would permit the evaluation of the titration method for accuracy and for applicability to banana analysis. If found to be applicable it would allow for subsequent studies concerning variations of vitamin C owing to stage of ripeness, to variety, to cooking treatment, etc., by means of the titration method without the necessity of a parallel bioassay. Such titration studies would then establish the true range of vitamin C variation in bananas.

In this bioassay the technique of Sherman, La Mer, and Campbell (1922) was used with one modification. The basal scorbutic diet contained two per cent of cod liver oil and eight per cent of butter fat

instead of the usual 10 per cent of butter fat. This modification was made to insure a liberal supply of vitamin D. The titration method used was essentially the Ahmad (1935) modification of the Bessey and King (1933) technique. The results of this experiment are shown (Table 4).

The behavior of the negative control group—all animals dying of severe scurvy between 20 and 45 days—indicated the correct anti-scorbutic properties of the basal ration. The positive control group

TABLE 4

Weight Gains and Scurvy Scores of Guinea Pigs Used in the Vitamin C Assay of Bananas

Group No.	Number of animals	Supplement	Amount supplement fed daily	Av. wt. at beginning of experiment	Av. wt. gain during 90-day experimental period	Range and average of scurvy scores
				gm.	*gm.*	
1.....	6	Ascorbic acid	0.5 mg.	279	301±32	1.2(0-2)
2.....	1	Orange juice (Positive control)	3.0 c.c.	250	384	0
3.....	5	Negative control	....	274	All died between 20th & 45th day	21.0(20-23)
4.....	6	Banana	5.0 gm.	280	324±31	2.0(0-8)
5.....	6	Banana	7.1 gm.	277	295±19	0.5(0-1)
6.....	6	Banana	10.0 gm.	282	296±16	0.3(0-2)

was included in this experiment merely for comparison purposes at autopsy.

The group of guinea pigs listed as receiving 7.1 grams of banana per day (Table 4) actually were fed varying amounts which were determined by titration to contain .5 mg. of ascorbic acid each day. Over the 90-day experimental period, however, these amounts averaged 7.1 grams per day.

Since five grams of banana produced a gain in body weight and average scurvy score comparable to .5 mg. of ascorbic acid, this quantity of banana pulp contains .5 mg. of ascorbic acid or 10 International vitamin C units and is considered a minimum protective dose. As commonly expressed this would be 57 International vitamin C units per ounce. It is regrettable that a lower level of banana pulp was not fed. From the close similarity of both weight gain and scurvy score between Groups 1 and 4, however, it seems probable that any amount less than five grams of banana would not give complete

enough protection from scurvy to be considered a minimum protective dose.

The fact that the groups receiving 7.1 and 10 grams of banana did not make significantly greater gains in weight than did the group receiving five grams, indicates merely that these amounts, 7.1 and 10 grams of banana, also contain at least .5 mg. of ascorbic acid. The superiority of Groups 5 and 6 over Group 4 apparently occurs entirely in improvement of the scurvy score.

The daily titration values indicated that an average of 7.1 grams of banana were necessary to furnish .5 mg. of ascorbic acid per day, whereas actually 5 grams of banana contained this amount of ascorbic acid. Thus it seemed probable that all of the physiologically potent vitamin C in bananas was not detected by the usual titration technique. Further work will be carried out to clarify this phase of the problem and will be reported later.

SUMMARY AND CONCLUSION

Standard bioassay techniques were employed to investigate the vitamin A, B_1, and C potency of ripe bananas. The fruit used was the banana of commerce in the United States.

Bananas were found to contain 71 to 95 International vitamin A units per ounce; four to five International vitamin B_1 units per ounce; and approximately 57 International vitamin C units per ounce.

The chemical titration technique apparently does not detect all of the physiologically active vitamin C present in bananas.

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THE SALT LIMITS AND THERMAL STABILITY OF A NEW SPECIES OF ANAEROBIC HALOPHILE

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(Received for publication, June 4, 1937)

Foremost of the group of halophilic bacteria of importance in industrial processes appear to be the chromogenic types described by Harrison and Kennedy (1922), Cloake (1923), Robertson (1931), and others. These organisms, which are often associated with color changes in salted fish and hides, have been shown by Harrison and Kennedy; Cloake; and Stuart, Frey, and James (1933) to be mainly derived from the solar salt used in the curing processes; their ultimate source is most probably sea water.

Morphologically, the majority of the halophilic organisms reported are cocci, although rod-shaped species have been noted by Lloyd (1929), Petter (1931), Lochhead (1934), and Bergey (1934); while Petrova (1929) reported a spore-forming rod isolated from solar salt. With reference to the oxygen relationships of halophiles Akimoto (1928) isolated strains from solar salt which grew in 25 per cent NaCl-media under anaerobic conditions, and Bergey records several aerobic strains that are facultative, but no obligate anaerobic species appear to have been reported.

ISOLATION AND TECHNIQUE

This communication deals with an anaerobic halophile obtained from Mediterranean salted anchovies. It has been isolated frequently from the fish muscle and the solar salt (the probable infecting agent) in which the fish is packed. The organism grows readily under anaerobic conditions in standard laboratory media containing 15 per cent NaCl. Isolation presented little difficulty, primary cultures in NaCl-cooked-meat medium developing within 7 to 10 days and secondary cultures becoming positive in 24 to 48 hours. Unless otherwise stated, all media used in this investigation contained 15 per cent of NaCl. NaCl-agar growth for microscopical examination was emulsified in 15-per cent, sterile NaCl solution after the manner of Clayton and Gibbs (1927).

DESCRIPTION OF *BACTERIODES HALOSMOPHILUS*

Morphology: 48 hours NaCl-agar. Rod-shaped cells with rounded ends, occurring singly, in pairs, and less often in chains.

Cells often curved and vary considerably in size and shape.
Average dimensions: .5 μ x 2.0-3.0 μ. (See Fig. 1.)
10 days NaCl-agar. Cells exhibit pleomorphism. Protoplasm becomes granular and vacuolated. (See Fig. 2.)

Endo-spores: Not present.

Motility: Negative.

Gram-stain: Negative. Cells stain unevenly, owing to plasmolytic changes.

NaCl-agar colony: Full development requires 4 to 6 days. Diameter 2 to 3 mm. Colonies finely granular, smooth, moist, low convex, round, and entire. They are translucent, buff-colored, and readily emulsified in 15 per cent NaCl solution. (See Fig. 3.)

NaCl-agar streak: Buff-colored, translucent, beaded.

NaCl-nutrient broth: Even turbidity with sparse, granular sediment.

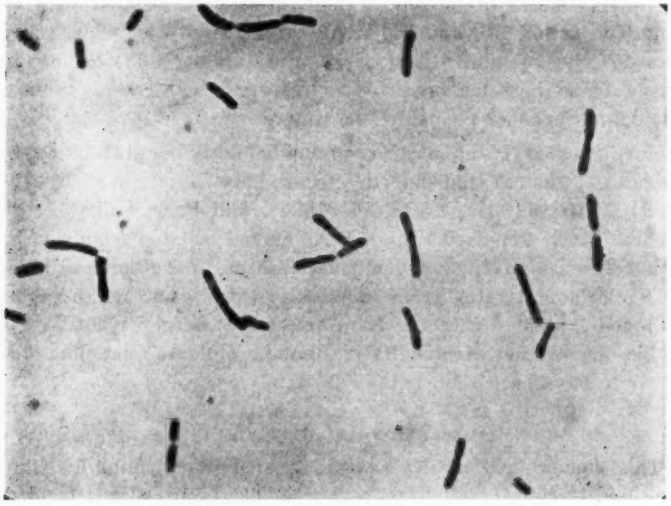

FIG. 1. X 2280 gram stain. 48 hours. NaCl-agar.

NaCl-cooked-meat medium: Gas produced. Meat slightly reddened in 4 days. Culture gives slight fetid odor. Analysis of gas:

H_2 = 87 per cent.
CO_2 = 5 per cent.
H_2S = Trace.

The remainder was inert gas.

NaCl-nutrient gelatin: No liquefaction in 21 days.

Milk: Not tested as NaCl adversely affected physical state of milk.

Indol: Nil in 21 days.

H_2S: Trace.

Starch hydrolysis: Nil in 21 days.

Nitrate reduction: Nil in 21 days.

Fermentation reactions: Acid and gas in dextrose, maltose, and glycerol. Fermentation is slow (i.e., about one week for production of acid and gas). Lactose, sucrose, inulin, mannitol, dulcitol, and salicin were not attacked.

Oxygen relationships: Obligate anaerobe.

Osmotic requirements: More than 4 per cent NaCl is required in the medium for growth to occur. Optimum 12½ to 15 per cent. Growth in 4 days in cooked-meat medium saturated with NaCl.

Temperature requirements: Optimum growth at about 35°C.(95°F.). At 22°C. (71.6°F.) there is slight growth in 4 to 5 days. No growth at 56°C.(132.8°F.).

pH Range for growth: Growth occurs between pH 5.5 and 8.5 in NaCl broth containing .001 per cent cystine HCl. Optimum—pH 7.4 to 7.6.

Source: Salted Mediterranean anchovies.

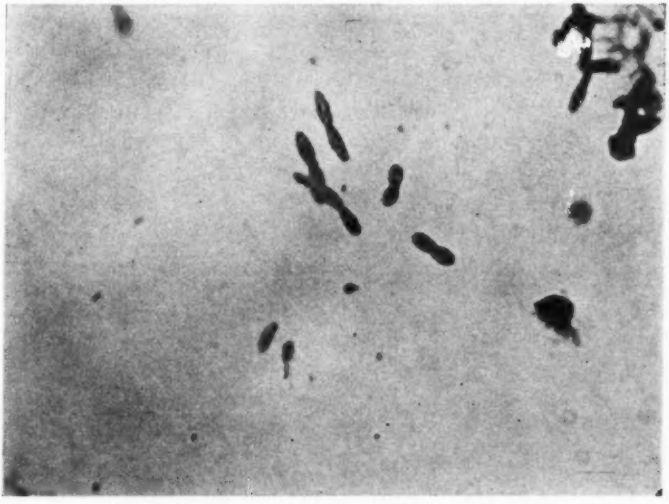

FIG. 2. X 2280 gram stain. 10 days. NaCl-agar.

CLASSIFICATION

The characteristics of the organism indicate that it is a member of the *Bacteriodes* group. The name *Bacteriodes halosmophilus* is proposed. Sub-cultures of the organism have been lodged with the National Collection of Type Cultures, The Lister Institute, London, England.

SALTS TOLERANCE

Richter (1928) states that Na, in relation to growth of *Bacterium phosphoreum* has two functions: one as a necessary nutrient and the other as a regulator of osmotic pressure. The latter function can be performed by any of a large series of salts.

With reference to the efficiency of the osmotic function of other salts, this has been confirmed in experiments with our organism, growth occurring when NaCl is replaced by any one of several other metallic chlorides. Experiments concerning nutritional function were not made, and it is emphasized that in the following experiments a small amount (about .3 per cent) of NaCl was supplied to the test medium with the inoculum and was thus available for nutritional purposes. The tests were made in cooked-meat medium containing equimolar concentrations of the salts. In all cases the pH of the medium was 7.4 to 7.6. After inoculation with .2 ml. of a four-day NaCl-broth culture per 10 ml. of test medium, cultures were incubated

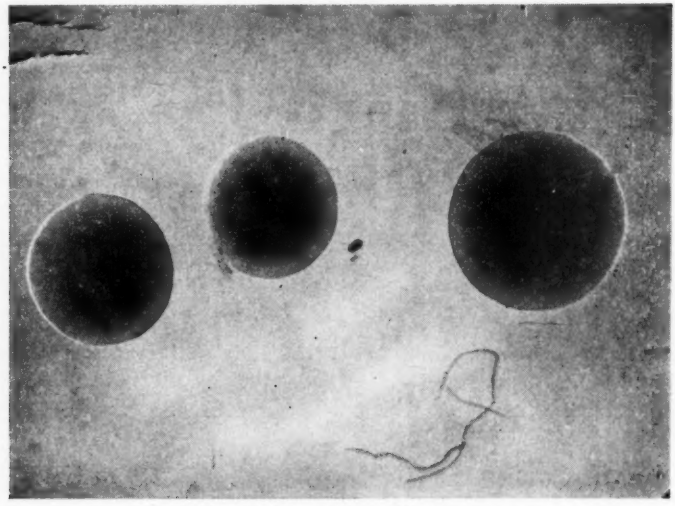

FIG. 3. X 10. 6 days. NaCl-agar.

anaerobically for a minimum of 21 days. The optimum concentration of NaCl was somewhat obscure but from consideration of the gas yield and degree of turbidity of the medium, was judged to be about 2.5 mol. At the optimum concentrations, growth in NaCl media was superior to the growth in any of the other series (Table 1).

The chlorides conform to a definite Hofmeister cation series in respect of the optimum concentrations required for growth, the amounts decreasing in the order — Na > K > Li > Mg > Ca (Nil). Growth did not occur in any concentration of NaBr. Rubentschik (1926) found that the toxic action on urea-splitting bacteria of salts expressed in equimolecular concentrations was $CaCl_2 > MgCl_2 > KCl > NaCl$. Gortner, Hoffman, and Sinclair (1928), in connection

with the peptisation of proteins, found a Hofmeister series of cations, the peptising action increasing: Na < K < Li < Mg < Ca. It is suggested that this may be of some significance in connection with the results of the growth experiments (Table 1) and Rubentschik's results.

HEAT RESISTANCE

With reference to the effect of NaCl in the heating medium on the thermal resistance of bacteria, Esty and Meyer (1922) found that spores of *Clostridium botulinum* showed a decreased resistance when heated in the presence of 8, 10, and 20 per cent NaCl, while from 1 to 2 per cent resulted in increased resistance. Viljoen (1926), working with spores of several types of bacteria, found that up to four per cent of NaCl increased their thermal resistance. Murray (1931) also showed that resistance to thermal destruction of bacterial spores was decreased with increasing salt concentration. In the following experiments the heat resistance was determined in McIlvaine's buffer solution (pH 7.0) containing 25, 15, 12.5 and 5 per cent of NaCl, these concentrations representing approximately the maximum, the optimum, and the minimum amounts required for growth. The salt had the effect of slightly lowering the pH of the buffer solution, in the 25-per cent concentration the pH changing from 7.0 to 6.8, but it was not considered likely that this would affect the result qualitatively. The bacterial emulsions, standardized to 850 million cells per milliliter with a Thoma cell were prepared from seven-day NaCl agar cultures. The precaution of washing the cells was not observed owing to the high specific gravity of the solutions. One-milliliter amounts in small glass ampuls were heated at 60 to 61°C.(140 to 141.8°F.) by the method of Williams (1929). Sub-cultures, made into NaCl-cooked-meat medium, were incubated anaerobically for a minimum of 21 days. Results are shown (Table 2).

The heat resistance of the organism varies in relation to the concentration of NaCl present in the heating medium. Minimum resistance is shown at the minimum concentration necessary for growth (five per cent), maximum resistance occurring in a concentration approaching the maximum for growth (25 per cent). It is assumed that the basic resistance is that shown when NaCl in the heating medium is present in a concentration equivalent to the optimum amount required for growth (12.5 to 15 per cent).

DISCUSSION

While our knowledge of the nature of the protein material in bacteria generally is very meager, there appears to be no published account of the protein character of salt tolerant or obligate halophilic

TABLE 1

Salts Tolerance of Halophilic Anaerobe

	Concentration (mol.)											
	0.25	0.5	0.75	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
NaCl	—	—	—	+[1]	+[1]	+[1]	+[1]	+[1]	+[1]	+[2]	+[3]	+[4]
KCl	—	—	—	—	+[2]	+[1]	+[2]	+[2]	+[4]	+[6]	—	—
LiCl	—	—	+[2]	+[1]	+[1]	+[2]	—	—	—	—	—	—
$MgCl_2$	—	+[1]	+[2]	+[2]	—	—	—	—	—	—	—	—
$CaCl_2$	—	—	—	—	—	—	—	—	—	—	—	—
NaBr	—	—	—	—	—	—	—	—	—	—	—	—

(Small superior figures denote day on which growth appeared.)

strains. It is obvious that very interesting problems arise in connection with the growth of microörganisms in concentrated salt media. Unfortunately little is yet elucidated respecting protein behavior in concentrated or saturated NaCl solutions.[1]

Attention has been drawn to this gap in the knowledge by Pauli and Valkó (1933), who state: "At present theory is very incomplete regarding the solubility of proteins as related to the presence of neutral salts in the solvent. Water-soluble and water-insoluble proteins may exhibit increased and decreased solubilities due to added salts. With a given protein and a given salt the solubility effect may be reversed according to the concentration of the salt."[2] They then discuss Debye's theory and conclude: "salting out will be more pronounced the more the protein reduces the dielectric constant of the solution." On this theory there is an additive ionic effect in salting out, cations and anions both being effective. In a certain sense this supports the Hofmeister ion series,[3] but the molecular structure of the particular protein involved also plays an important role.[4]

SUMMARY

A new species of halophile of the Bacteroides type was isolated from salted anchovies. The name *Bacteriodes halosmophilus* is proposed for this organism which is an obligate anaerobe.

Maximum growth occurs when the NaCl concentration of the medium reaches 12½ to 15 per cent. Growth does not occur with four per cent, or less, NaCl present. As a regulator of osmotic pressure, NaCl is replaceable by several other metallic chlorides which form a Hofmeister cation series in respect of the concentrations showing maximum growth: Na > K > Li > Mg > Ca.

The heat resistance of the organism in the presence of NaCl varies in relation to the NaCl concentration, maximum resistance occurring in a concentration approaching the maximum amount allowing growth; minimum resistance is shown at the minimum concentration allowing growth.

[1] Staker, E. V., and Gortner, R. A., 1931. J. Phys. Chem. **35**, 1565, in a lengthy account of the peptisation of vegetable proteins by various salt solutions discuss many of the difficulties of the problem.

[2] Cf. Sandor, G., Bonnefoi, A., and Perez, J. J., 1933. Precipitation of proteins by neutral salts. Compt. rend, **197**, 1245. Bonot, A., 1934. The solubility of serum proteins in concentrated solutions of neutral salts. J. chim. phys. **31**, 301.

[3] See Bancroft, W. D., and Gould, L. P., 1934. The hydrols. J. Phys. Chem. **38**, 197.

[4] Haurowitz, F., 1936. Ionenstruktur, Löslichkeit und Flockung der Proteine. Kolloid-Z. **74**, 208, relates the solubility to the electric charge distribution in the protein molecule, i.e., surface or internal charges.

TABLE 2

Effect of NaCl on Heat Resistance of Halophilic Anaerobe

Concentration of NaCl in McIlvaine's buffer (pH 7.0)	Heating time in minutes. Temperature 60-61°C.(140-141.8°F.)															
	2½	5	7½	10	12½	15	17½	20	25	30	35	40	45	50	55	60
5 %.....	$+^{2}$	$+^{4}$	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12.5%.....	$+^{2}$	$+^{2}$	$+^{2}$	$+^{3}$	$+^{3}$	$+^{4}$	—	—	—	—	—	—	—	—	—	—
15 %.....	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{4}$	$+^{4}$	$+^{4}$	—	—	—	—	—	—	—	—
25 %.....	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{3}$	$+^{3}$	$+^{3}$	$+^{4}$	$+^{4}$	$+^{4}$	$+^{5}$	$+^{6}$	—	—

(Small superior figures denote day on which growth appeared.)

Attention is drawn to a serious gap in the knowledge of bacterial proteins—the influence on solubility of concentrated salt solutions.

The author wishes to thank Dr. William Clayton for his helpful criticism and advice.

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VITAMIN C IN CITRUS-JUICE BEVERAGES AND CANNED GRAPEFRUIT JUICE

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(Received for publication, June 19, 1937)

The importance attached to citrus juice as a source of vitamin C has led to considerable research on fresh and canned juice as well as on the dairy type of citrus-juice beverages, especially since the advent of chemical methods for the estimation of the antiscorbutic vitamin.

In their report on the vitamin-C content of dairy orange beverages, Mack, Fellers, MacLinn, and Bean (1936) furnished some valuable information on this subject, but it was thought that data supplementing their work with other brands and types of citrus drinks would be of interest.

The desirability of further study on the loss of vitamin C in canned grapefruit juice over long periods of storage has prompted the phase of this report dealing with canned juice.

Dairy-type orange beverages are usually prepared by adding sugar and water to concentrated or to unconcentrated orange juice. Such drinks are dispensed in milk bottles usually with a cardboard cap. Carbonated orange beverages may be prepared either by adding carbonated water to concentrated orange juice, or by adding essential oils, sugar syrup, color, and fruit acids to carbonated water.

Methods used for processing grapefruit juice for canning have been adequately described by Stevenson (1934).

EXPERIMENTAL PROCEDURE

According to Bessey and King (1933) citrus juice lends itself more suitably to the determination of vitamin C by chemical methods than do certain other plant and animal materials, but there are other reducing agents present in the juice which also react with the reagents. Lemon juice is influenced to a lesser degree by these interfering substances than either grapefruit or orange juice. Owing to the overwhelming ratio of vitamin C to the other reducing agents, however, results obtained by chemical methods are rendered sufficiently reliable.

The 2,6,dichlorophenolindophenol method of Tillmans, Hirsch, and Hirsch (1932), modified by Bessey and King, was compared with the iodine method of Reynolds and Stevens (1934) by Mack and associates (1936) who found that of the two chemical methods, the iodine

TABLE 1

Analysis of Citrus-Juice Beverages

Sample No.	Name of product	Contains juice of	Total solids	Citric acid	Ratio	Ascorbic acid	Vitamin C	Ascorbic acid[1]	Vitamin C[1]	Loss
			pct.	*pct.*		*mg./100 ml.*	*units per oz.*	*mg./100 ml.*	*units per oz.*	*pct.*
1	Green Spot	Orange	13.4	0.31	43.2	1.42	8.42	0.89	5.28	37.3
2	Jus-Rite	Orange	12.8	0.77	16.6	27.8	165	19.8	118	28.3
3	Jus-Rite	Tangerine	15.2	0.72	21.2	14.8	88.0	12.1	71.8	18.2
4	Florida Citrus Juice Co.	Orange	13.1	0.96	13.7	38.2	227	35.4	210	7.3
5	Florida Citrus Juice Co.	Grapefruit	11.3	1.03	11.0	16.5	97.8	9.60	57.0	41.8
6	Don's Tru-Fruit	Orange	13.2	0.53	24.9	12.1	71.9	7.70	45.7	36.4
7	Don's Tru-Fruit	Grapefruit	11.7	0.48	24.4	3.56	21.2	1.10	6.53	69.1
8	Bireley's	Orange	10.8	0.35	30.8	1.95	11.6	1.10	6.53	43.6
9	Orangeade from booth at a fair	.....	N.D.[2]	0.15	N.D.	0	....	....	....	....
10	Nehi	Orange	14.0	0.24	58.3	0.89	5.28	N.D.	N.D.	N.D.
11	King's Court Sour (C)[3]	Lemon	10.4	0.32	32.5	0.36	2.14	N.D.	N.D.	N.D.
12	Nu-Icy (C)	Orange	15.7	0.10	157.0	0	....	....	....	....
13	Celo (C)	Orange	15.8	0.12	131.7	0	....	....	....	....

(Not stated to contain juice)

[1] One week later. [2] N.D.=not determined. [3](C)=carbonated.

Florida Valencia oranges .45 mg. per c.c. (267 units per oz.).

Florida pineapple oranges .51 mg. per c.c. (315 units per oz.)—Daniel, *et al.* (1936).

titration gave more constant and easily reproducible results. Joslyn, Marsh, and Morgan (1934) state that the iodine titration is superior in practice because it is easier to carry out and can be more readily duplicated. The iodine method was therefore deemed satisfactory and was used throughout in the present work.

Beverage samples were obtained direct from the retail dealers with bottles sealed in the usual way with metal or cardboard caps. Total solids, citric acid, and ratio of total solids to citric acid and vitamin C (ascorbic acid) were determined immediately, and the remainder of each sample was stored at 7°C.(44.6°F.) for one week, after which vitamin C was again determined. Carbonated beverages were treated in the same way except for removal of carbon dioxide by gentle heat.

Several canning plants were visited early in 1936, and samples of canned grapefruit juice were obtained. In order to secure the best assurance possible that the individual cans within each sample would contain practically the same amount of vitamin C at the time of canning, each sample consisted of juice from only one lot. Vitamin C was determined one or two days after canning and thereafter at intervals of one month, except for the unavoidable interval between July, 1936 and January, 1937.

DISCUSSION OF RESULTS

As a means of comparing the results (Table 1) with fresh orange juice, the figures reported by Daniel, Kennedy, and Munsell (1936) were deemed satisfactory, especially since they agreed remarkably well with the findings of this laboratory. For Florida Valencia oranges the value reported was .45 mg. per c.c., and for Florida pineapple oranges .51 mg. per c.c. (267 and 315 units per ounce, respectively).

The orange and grapefruit beverages analyzed contained from 2 to 85 per cent as much vitamin C as the average Florida Valencia orange. The best carriers found were the products of the Florida Citrus Juice Company, the Jus-Rite, and Don's Tru-Fruit beverages. The carbonated Celo orange was not stated to contain orange juice and could not be expected to contain vitamin C. Sample nine, which contained no trace of vitamin C, was obtained from a booth on a fair midway. It was attractively displayed in a large bowl and was stated to contain orange juice, sugar, and water. If the presence of vitamin C, however, is an indication of the presence of orange juice, the beverage was entirely synthetic.

Although flavors were well retained for the period of storage, losses of vitamin C varied from 7.3 to 69.1 per cent. Ratios of total

solids to percentage of citric acid were generally excessive as compared with fresh juice. A sample of Orange-Crush beverage was not obtainable, but was reported by Koch and Koch (1932) to contain only six to seven units of vitamin C per ounce.

The data for canned grapefruit juice were plotted (Figs. 1 to 6). No attempt was made to place all samples from each packer on one graph, the principal object being to avoid confusion in reading by placing two curves which were reasonably far apart on each figure; for example, Fig. 1 represents a sample of sweetened juice from Packer A and also a sample of sweetened juice from Packer D. In every sample the obvious trend of vitamin-C content is downward, the two extremes being presented in Fig. 3, which represents a sample of sweetened juice from Packer C and a sample of the drained liquid from canned grapefruit sections also from Packer C.

While the results reported are not in exact agreement with those of Fellers and Isham (1932), who found that "commercially canned orange juice, orange slices, grapefruit juice or grapefruit slices after nine months' storage contained fully as much vitamin C as fresh fruits," the differences may perhaps be explained by the fact that their canned citrus products had already been in storage from seven to ten months when examined. The results here reported, however, indicate an average loss of only 25 per cent at termination of experiments (nine to 15 months), which is probably not of sufficient magnitude to alter the statement of Fellers and Isham to the effect that canned grapefruit products are excellent sources of vitamin C.

SUMMARY AND CONCLUSIONS

Eight samples of dairy-type citrus beverages examined for ascorbic acid by the iodine method showed ascorbic-acid values from 1.42 to 38.2 milligrams per 100 milliliters, corresponding to from 8.42 to 227 units of vitamin C per ounce. A sample of orangeade obtained from a booth at a fair contained no vitamin C. The carbonated citrus beverages, of which four were examined, contained from zero to .89 milligrams of ascorbic acid per 100 milliliters, corresponding to from zero to 5.28 units of vitamin C per ounce. For comparison with fresh juice the values 267 and 315 units per ounce were used.

Loss of vitamin C in dairy-type beverages is rapid even at cold-storage temperature.

Ratios of total solids to citric acid are generally high as compared with fresh juice.

Twelve samples of grapefruit juice from five different packers were examined monthly for loss of vitamin C and were found to

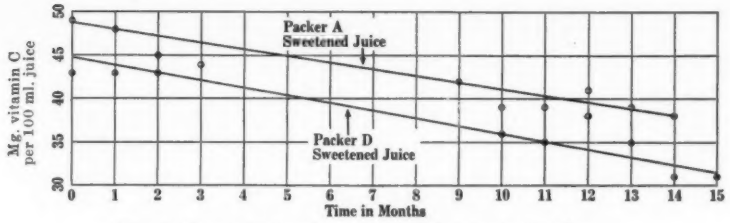

FIG. 1. Loss of vitamin C in canned grapefruit juice.

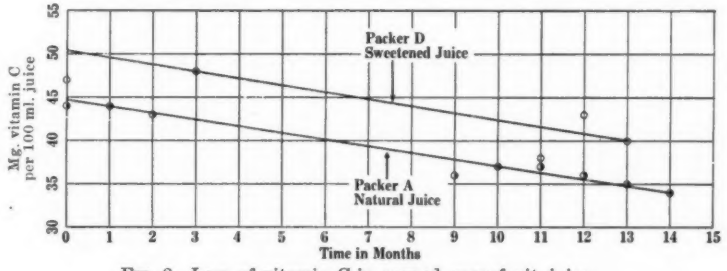

FIG. 2. Loss of vitamin C in canned grapefruit juice.

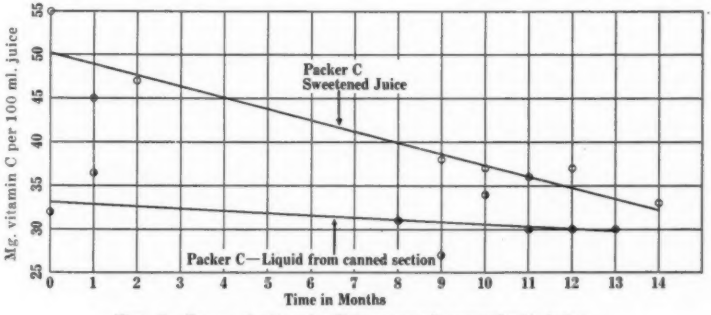

FIG. 3. Loss of vitamin C in canned grapefruit juice.

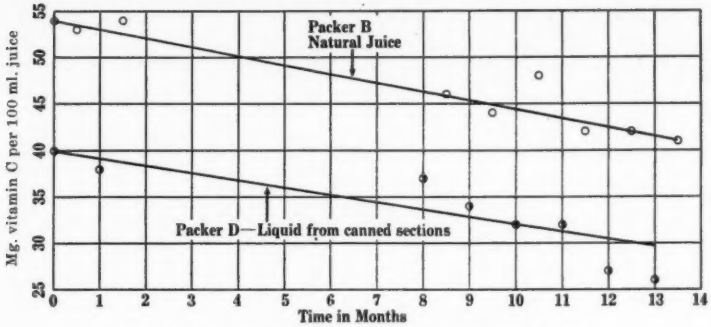

FIG. 4. Loss of vitamin C in canned grapefruit juice.

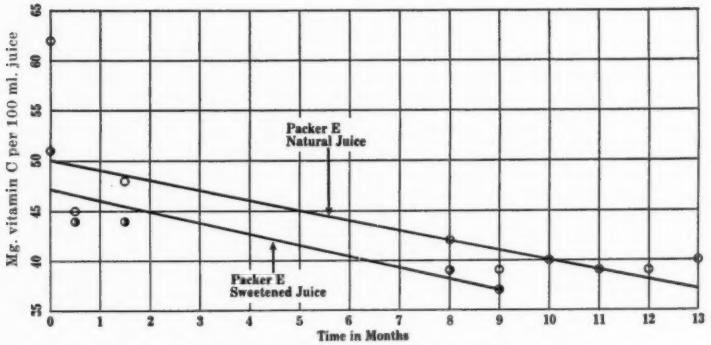

FIG. 5. Loss of vitamin C in canned grapefruit juice.

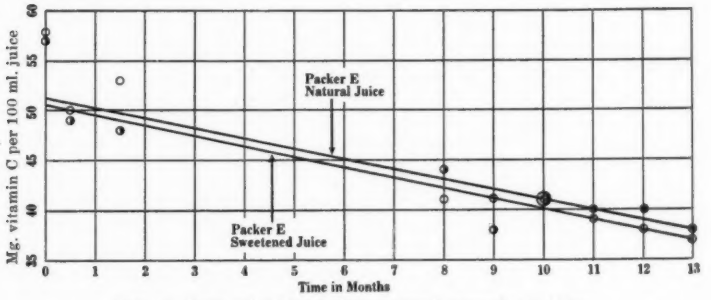

FIG. 6. Loss of vitamin C in canned grapefruit juice.

Key to grapefruit canners whose products are plotted in Figs. 1 to 6

Packer	Name and Address
A	Florida Citrus Canners Coöperative, Lake Wales, Fla.
B	DeSoto Canning Company, Arcadia, Fla.
C	West Coast Fruit Company, Clearwater, Fla.
D	Floridagold Citrus Corporation, Lake Alfred, Fla.
E	Florida Fruit Canners, Inc., Frostproof, Fla.

have lost an average of 25 per cent at the end of from nine to 15 months.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to L. Longfield-Smith, formerly connected with this department, who began the experiments on canned grapefruit juice; and to H. W. von Loesecke of the Bureau of Chemistry and Soils, United States Department of Agriculture, for his helpful suggestions and criticisms during the preparation of this manuscript.

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EFFECT OF FREEZING AND THAWING BEEF MUSCLE UPON PRESS FLUID, LOSSES, AND TENDERNESS[1]

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(Received for publication, June 24, 1937)

INTRODUCTION

The use of frozen meat is growing constantly in the United States. Commercially, frozen storage is much employed to balance periods of supply and demand. Freezer storage is becoming increasingly available through the development of the practice among cold-storage plants of renting freezer lockers where meat and other foods may be stored.

Much research has been conducted on the freezing and thawing of meat, but there has been very little beyond testing the raw meat. To furnish information concerning cooked beef frozen prior to cooking, a study was undertaken of the effect of freezing and of various thawing temperatures on thawing and cooking losses, drip, press fluid, total moisture, and tenderness.

REVIEW OF LITERATURE

In considering the effect of freezing on meat, the change in the structure caused by freezing is important in determining how nearly the meat will resemble unfrozen meat when thawed. Moran (1929) found that on freezing muscle, centers of crystallization are formed which are most active between the fibers. With normal cooling the fibers shrink, become distorted, and are separated by masses of ice. Cook *et al.* (1926) found that a much greater amount of ice forms within the individual fiber than without, making it appear that the alteration of the colloidal structure commences within the fiber.

Plank (1925) points out the importance of two effects of freezing: the damage to the protoplasmic structure by formation of ice crystals and the consequent dehydration of the colloid. The denaturation of the proteins of meat, maximal in the range —2 to —3°C. (28.4 to 26.6°F.), is stressed by Moran (1935). Work on haddock by Sherrill (1928) shows that slow freezing damages the muscle structure, the sarcolemma being ruptured. Vickery (1926) also emphasizes the importance of the effect of freezing on the sarcolemma, his work indicating that the sarcolemma has an important role in determining the amount of reconstitution which will take place.

[1]Published with the approval of the Director as paper No. 1520, Scientific Journal Series, Minnesota Agricultural Experiment Station.

The thawing of frozen colloidal systems may not give restoration of the prefrozen state. Moran (1925) points out that with systems where recovery is not complete there exists a certain limited range of temperature (critical range) within which the separation process may occur. The change of state may occur either during the fall or during the rise of temperature.

Drip is defined by Cook *et al.* as the clear, reddish-colored fluid which exudes from all cut surfaces of meat that has been frozen and thawed. The presence of drip has been shown to be due to changes which have taken place in the meat during the freezing and thawing. Empey (1933) studied the effect on drip of rates of freezing and thawing, age of animal, length of period between slaughter and freezing, period in frozen state, sex and breed, and composition of muscle including pH. Of these, he found pH to be the only important factor, for a definite relationship existed between the pH of the muscle and the susceptibility of the thawed muscle to drip.

Moran and Hale (1932) found that time of storage at any one temperature had little effect on the amount of drip, but that the temperature of storage made a difference. Using 10°C. (50°F.) for rapid thawing, and 1°C.(33.8°F.) for slow thawing, they found no variation in drip. Reay (1934), working on haddock, found that the determining factor for the amount of drip is the time spent in the range —1 to —5°C.(30.2 to 23°F.) either during freezing or thawing; and he considers the drip formed within the first hour of exposure to this range to be due to structural changes caused by the formation of ice, attributing the slow increase in drip with exposure up to 10 days to development of denaturation of the proteins. The conditions found by Cook *et al.* to affect the amount of drip were the rate of freezing, the rate of thawing, the time of storage after thawing, storage before freezing, and possibly the temperature to which the meat was reduced in the freezing process. Of these factors the first appeared to be the most important.

MATERIALS AND METHODS

Two problems were investigated: (1) the difference between frozen roasts thawed at different temperatures and (2) the difference between unfrozen and frozen roasts. Medium-grade steers, as uniform as possible, were selected at a local packing house. The cuts used were the 9-10 and 11-12 rolled rib roasts, which were ripened 10 days. The 9-10 rib roasts averaged 3.6 pounds and the 11-12 rib roasts 2.8 pounds in weight. Twelve pairs of roasts were used. Losses were determined for the entire roast. All other determinations were made on the longissimus dorsi muscle which forms the eye muscle of the 9-10 and 11-12 rib roasts.

Freezing was done in an ice cream cabinet which maintained a temperature of —18°C.(—0.4°F.). Each roast was weighed, a thermometer inserted so that the center of the bulb came to the center of the longissimus dorsi muscle, and each roast was wrapped in moisture-proof paper to prevent excessive drying-out during freezing. The average time required for the internal temperature of the roast to fall to —18°C. was 25 hours.

For thawing, each roast was unwrapped, weighed, and placed on a rack 1.25 cm. high in a weighed sheet-iron pan measuring 20 by 25.25 by 6.25 cm. The roasts to be thawed at 175°C.(347°F.) were placed in an electric oven preheated to 175°C. and removed when the thermometer registered 5°C.(41°F.). The time for this averaged 35.06 minutes per pound. The roasts to be thawed at 24-25°C.(75.2-77°F.) were placed in a conditioning room where the temperature varied from 24 to 25°C. and the humidity was held constant at 65 per cent. The roasts were removed when the thermometer registered 5°C., requiring an average of 187.96 minutes per pound. The thawing end-point, 5°C., was selected because that is the upper limit of the critical range of cooling specified by Moran (1932).

Cooking was done in electric ovens having a temperature control accurate to ±1°C.(±1.8°F.). The methods of the cooking committee of the coöperative meat investigation committee, Alexander, Clark, and Howe (1933), were followed. The roasts were cooked uncovered to 58°C.(136.4°F.) in a 175°C. oven. Losses were determined by loss of weight of the meat and weight of the drippings collected.

SAMPLING

The roasts were allowed to come to maximum interior temperature before cutting. The longissimus dorsi muscle was cut through the thermometer hole across the grain of the meat. A slice 1.25 cm. wide was cut from the left half of the muscle. From this slice five samples were taken with a cylindrical borer having a diameter of 1.25 cm. Two of the samples were taken from the center of the muscle, one on each side of the thermometer hole, the other three from the bottom of the muscle. The two taken at the thermometer hole were used for press fluid, the other three for drip determination. The remainder of this slice was used for total moisture determination. Two samples for tenderness determination were cut from the right half of the muscle, using a cylindrical borer one inch in diameter.

DETERMINATION OF PRESS FLUID

Press fluid was determined by the method of Child and Baldelli (1934). The press fluid samples, immediately upon cutting, were

placed in tin foil cups in weighing cans and weighed. Each sample was wrapped in a cloth cross, placed in the tray of the pressometer, and subjected to 250 pounds pressure for 10 minutes. The sample was removed from the cloth wrapping and again weighed. The amount of press fluid is expressed as percentage of weight lost on the basis of the original weight of the sample.

DETERMINATION OF DRIP

The method for determination of drip was that of Cook, Love, Vickery, and Young (1926), using absorption by blotting paper. The samples cut for drip estimation were placed immediately in tin foil cups in weighing cans, weighed, then placed on blotting paper and covered with blotting paper held in place by light pressure (approximately six grams per square centimeter). The samples were covered with small boxes to prevent excessive drying owing to evaporation, and left for two hours. At the end of this time the samples were replaced in the weighing cans and weighed again. The difference is considered drip and is expressed as percentage of the weight of the original sample.

DETERMINATION OF TOTAL MOISTURE

The remainder from the slice sampled for press fluid and freezing drip was divided between two weighing cans and weighed. Each sample was cut fine with scissors and placed in a weighing can having a piece of filter paper in the bottom. The cans were placed uncovered in a drying oven at 60°C.(140°F.) for two hours, then dried at 100°C. (212°F.) in a Freas vacuum oven maintaining a pressure of 2 to 2.5 cm. of mercury. At the end of five hours the cans were removed, covered, cooled, and weighed. The weight lost during drying is considered total moisture and is expressed as percentage of the original weight of the sample.

DETERMINATION OF TENDERNESS

Tenderness was measured by means of the Minnesota Shear Stress apparatus. Each sample consisted of a cylinder of meat 1 inch in diameter and 2 to 2.5 inches long. The sample was sheared through the center, then each of the resulting pieces sheared through the center again, giving three readings for each sample and six for each roast. The tenderness is given as force in pounds necessary to shear the sample.

STATISTICAL METHOD

The t test given in Fisher (1932), section 24, was used to test the significance of the difference between means. Any t value having a probability equal to or less than .05 was considered significant.

EFFECT OF THAWING TEMPERATURES

A comparison was made of the effect of different thawing temperatures [175°C.(347°F.) and 24-25°C.(75.2-77°F.)] on losses, press fluid, drip, total moisture, and tenderness of cooked beef which had been frozen prior to cooking.

The 9-10 rolled rib roasts were placed in the freezing cabinet and remained 30 hours. They were then removed. The roasts from the left side of each pair thawed at 175°C., those from the right side at 24-25°C., humidity 65 per cent. On completion of thawing the roasts were cooked at 175°C. to 58°C.(136.4°F.), allowed to come to maximum temperature, and sampled.

The results indicate that the different thawing temperatures used do not affect press fluid, drip, total moisture, or tenderness of beef (Table 1). The total losses, including the freezing, thawing, and cooking losses, are significantly greater if the roasts are thawed at 175°C. than if they are thawed at 24-25°C. This is due, at least partially, to the fact that the roasts thawed at 24-25°C. gain in weight during thawing, while those thawed at 175°C. lose weight (Table 2).

EFFECT OF FREEZING

To show the effect of freezing a comparison was made of frozen and unfrozen beef after cooking, in which losses, press fluid, drip, total moisture, and tenderness were studied.

The left-side, unfrozen, rolled 11-12 rib roasts were cooked at 175°C.(347°F.) to 58°C.(136.4°F.), allowed to come to maximum internal temperature, then sampled. The right-side roasts were placed in the freezing cabinet at the same time the left-side roasts were cooked, remained there 72 hours, were thawed at 175°C., cooked at 175°C. to 58°C., allowed to come to maximum interior temperature, and sampled.

The frozen beef had significantly greater total losses than the unfrozen, and significantly less press fluid. The differences between the frozen and unfrozen beef for drip, total moisture, and tenderness were insignificant (Table 3).

CONCLUSIONS

As a result of statistical analysis of the data obtained the following conclusions were reached.

Unfrozen beef has a significantly lower total loss than frozen beef. Frozen beef thawed at 175°C. has a higher total loss than frozen beef thawed at 24-25°C. Frozen beef thawed at 24-25°C., humidity 65 per cent, gains weight rather than losing it by evaporation as does frozen beef thawed at 175°C.

TABLE 1

Total Losses, Press Fluid, Drip, Total Moisture, and Tenderness of Frozen Beef Thawed at 175°C.(347°F.) and at 24-25°C.(75.2-77°F.) and Cooked at 175°C. to 58°C.(136.4°F.)

Pair No.	Total losses [1]		Press fluid		Drip		Total moisture		Tenderness	
	175°C.	24-25°C.	175°C.	24-25°C.	175°C.	24-25°C.	175°C.	24-25°C.	175°C.	24-25°C.
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*lbs. force*	
1	19.65	17.84	39.38	41.28	13.56	14.64	67.02	64.89	31.25	29.92
2	19.75	18.98	41.70	40.33	15.62	15.54	68.98	68.19	24.09	26.42
3	19.60	19.04	46.82	44.53	16.71	17.20	68.09	67.80	22.75	24.34
4	17.86	16.08	49.98	51.88	15.33	16.84	71.45	72.50	21.96	22.54
5	18.85	17.70	41.82	42.02	14.16	12.77	66.85	62.90	22.59	26.67
6	21.32	19.12	37.71	39.96	14.03	16.04	67.83	65.37	21.71	26.00
7	20.63	18.27	41.94	51.53	15.74	20.44	68.15	70.10	18.88	20.38
8	20.61	18.31	50.65	45.83	16.97	19.17	69.48	70.04	24.25	20.96
9	19.04	15.61	45.82	43.04	17.29	13.34	68.17	67.52	32.96	28.63
10	18.45	16.16	44.57	45.75	16.47	19.69	69.14	67.77	25.42	21.29
11	26.23	20.51	39.81	42.91	16.09	13.81	67.38	69.16	24.50	19.46
12	22.96	22.43	43.71	40.69	16.51	15.28	67.72	68.63	18.75	27.00
Mean	20.41	18.34	43.66	44.15	15.71	16.23	68.36	67.90	24.09	24.47
Mean difference	2.08		0.49		0.52		0.45		0.38	
t	5.00		0.44		0.54		0.86		0.32	
P	<0.05		>0.60		>0.60		>0.40		>0.70	

[1] Total losses are the sum of the evaporation loss during freezing and evaporation and dripping losses during thawing and cooking.

Unfrozen beef has a significantly higher press fluid content than frozen beef thawed at 175°C. Thawing temperature does not affect the press fluid content of frozen beef.

Total moisture, drip, and tenderness of cooked beef are unaffected by freezing or by different thawing temperatures.

TABLE 2

Percentage Evaporation Loss During Thawing of Frozen Beef Thawed at 175°C. (347°F.) and at 24-25°C.(75.2-77°F.), Humidity 65 per cent, Cooked at 175°C. to 58°C.(136.4°F.)

Pair No.	Thawing temperature	
	175°C.	24-25°C.
	pct.	*pct.*
1	4.19	—2.60
2	3.78	—2.32
3	5.60	—1.98
4	4.93	—2.17
5	5.11	—2.57
6	5.83	—2.35
7	5.82	—2.76
8	5.98	—2.75
9	4.49	—2.32
10	4.68	—2.44
11	6.74	—2.66
12	5.34	—2.81
Mean	5.21	—2.48
Mean difference	7.69	
t	27.85	
P	<0.01	

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TABLE 3

Total Losses, Press Fluid, Drip, and Total Moisture in Percentage and Tenderness in Pounds Force for Shearing, of Frozen Beef Thawed at 175°C.(347°F.) and Unfrozen Beef, Cooked at 175°C. to 58°C.(136.4°F.)

Pair No.	Total losses [1]		Press fluid		Drip		Total moisture		Tenderness	
	Frozen	Unfrozen	Frozen	Unfrozen	Frozen	Unfrozen	Frozen	Unfrozen	Frozen	Unfrozen
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*lbs. force*	
13	19.55	12.47	40.10	48.46	14.84	18.96	66.38	69.60	24.96	31.21
14	17.17	11.53	43.68	47.98	19.13	20.12	72.08	71.72	27.21	28.46
15	18.84	16.49	46.94	47.13	15.53	13.73	68.96	70.01	25.00	15.71
16	17.66	12.57	42.94	50.16	16.24	15.59	72.91	73.21	13.92	21.13
17	18.35	12.07	41.72	44.00	14.70	18.95	64.91	68.65	20.38	27.17
18	19.19	15.21	42.04	46.71	17.08	18.35	68.15	68.53	20.34	18.79
19	19.33	15.02	44.64	46.79	16.15	20.90	66.23	69.42	22.13	18.54
20	15.79	15.87	49.31	49.31	24.44	17.42	70.90	69.72	21.25	21.79
21	16.86	14.75	49.52	44.17	21.97	18.18	68.61	67.02	25.50	24.30
22	16.28	13.97	43.83	45.52	18.11	18.36	67.61	67.18	27.63	32.50
23	18.81	17.52	46.28	49.19	18.04	19.38	69.84	71.45	12.92	22.25
24	20.50	17.63	38.14	47.99	14.92	18.81	69.33	70.70	22.33	16.00
Mean	18.19	14.59	44.10	47.28	17.10	18.23	68.83	69.77	21.96	23.15
Mean difference	3.60		—3.19		—1.13		—0.94		—1.19	
t	5.76		2.68		0.87		1.86		0.71	
P	<0.01		<0.05		>0.40		>0.05		>0.40	

[1] Total losses are the sum of evaporation loss during freezing and evaporation and dripping losses during thawing and cooking for frozen roasts, and the sum of evaporation and dripping losses during cooking for unfrozen roasts.

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ISOLATION AND CULTURAL DIFFERENTIATION OF FOOD-POISONING STAPHYLOCOCCI

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(Received for publication, June 29, 1937)

Although methods are available for testing the gastroenterotoxic properties of staphylococci, it is generally agreed that food-poisoning strains cannot be differentiated with certainty from other types of staphylococci. For this reason, preliminary diagnosis of staphylococcus food poisoning depends upon finding this organism in increased numbers in the suspected food or vomitus. Where only a small number is present, the interpretation is difficult and it becomes necessary to rely on various tests, most of which have not proved entirely satisfactory.

It will be shown in this paper that food-poisoning staphylococci possess properties in common with other pathogenic staphylococci which make it possible to isolate them by the method of Chapman, Lieb, and Curcio. In addition, food-poisoning staphylococci possess characteristic properties which are useful for making a rapid presumptive diagnosis.

Several factors have complicated the investigation of food-poisoning staphylococci: (1) Only a small number of strains has been available for investigation. (2) There is no assurance that all "strains" of staphylococci which have been considered the cause of food-poisoning outbreaks represent the original gastroenterotoxic strains or that they are genetically related to them. (3) Stock strains have been treated as if their properties remain unaltered indefinitely. It will be shown that many of them contain dissociants which may be regarded as degenerate forms of the parent strain and which, when present in excess, may alter the properties of the strain. (4) Testing for gastroenterotoxic properties has not been entirely satisfactory and has been confused by factors mentioned in (3). (5) It is possible that other pathogenic staphylococci, not involved in food-poisoning outbreaks, may possess gastroenterotoxic properties.

The 56 food-poisoning cultures used in this investigation were obtained through courtesy of the following investigators, to whom the authors are deeply indebted for their helpful coöperation:

Dr. H. D. Pease, Pease Laboratories, New York City
1. Strain 178 isolated December 1935, from vomitus.

Dr. R. V. Stone, Los Angeles County Health Department, Los Angeles, California

2. Strain S 70-2 isolated November 30, 1935, from vegetable soup meat stock, six persons affected. Well-controlled epidemiology.
3. Strain S 82 isolated August 14, 1936, from baked ham. Sporadic cases.
4. Strain S 84-S isolated September 23, 1936, from chicken giblets; 26 persons affected. Kupchik's results were obtained with Strain S 84-B.
5. Strain S 26-D isolated February 13, 1935, from the vomitus of a female victim. About 100 persons were affected.
6. Strain S 27 isolated from a male victim of the same outbreak as Strain S 26-D.
7. Strain S 36 isolated February 26, 1935, from hollandaise sauce; 11 to 14 persons were affected.
8. Strain S 49 isolated April 20, 1935, from a cow. The udder was normal and there was no pus. Two families were affected. The first family owning the cow had "dysentery" and sold the cow because the milk did not agree with them.
9. Strain S 51 isolated May 9, 1935, from the same cow as S 49.
10. Strain S 53-4 isolated May 15, 1935, from canned chicken soup and canned milk combination. Several members of one family were affected. This strain was obtained from a Stone negative colony of Strain S 53-1 which gave a ++++ Stone reaction.
11. Strain S 87-S isolated October 14, 1936, from boiled smoked tongue; affected about 12 persons. Strain S 87-L was Dolman positive (Meyer).
12. Strain S 3 isolated November 9, 1934, from evaporated milk. About 100 persons were affected. This strain was obtained from a can other than the one suspected, but the serial number was the same.
13. Strain S 10 isolated December 10, 1934, from raw milk. About 125 persons were affected. A similar organism was isolated from the cow (Stone).

Dr. Luther Thompson, Mayo Clinic, Rochester, Minnesota

14. Strain Iowa. No data received.

Dr. Fred W. Tanner, University of Illinois, Urbana, Illinois

15. Strain C/3 isolated March 31, 1936, by Dr. G. A. Denison, Jefferson County Board of Health, Birmingham, Alabama, from cream puffs.
16. Strain B/1 isolated April 2, 1936, from the same outbreak; 122 persons were affected.
17. Strain McB/3 isolated in 1932, by Dr. R. A. McBurney, University of Alabama; 150 persons were affected.
18. Strain c-2 isolated by Dr. G. A. Denison from cream puffs.
19. Strain c-3 from the same outbreak but different puffs.
20. Strain c-4 from the same outbreak but different puffs.
21. Strain c-6 from the same outbreak but different puffs.
22. Strain R-1 from the same outbreak but different puffs.
23. Strain R-2 from the same outbreak but different puffs.
24. Strain R-7 from the same outbreak but different puffs.

Dr. G. M. Dack, University of Chicago, Chicago, Illinois

25. Strain 1 isolated December 1929, from Christmas sponge cake. See J. Prev. Med. **4**, 167, 1930.
26. Strain 9 isolated March 1931, from chicken gravy. See J. Prev. Med. **5**, 387, 1931.
27. Strain 53 isolated November 1930, from devil's food cake. See Food Research **1**, 237, 1936.

28. Strain 95 isolated April 1932, from cake filling. See Am. J. Hyg. **20**, 604, 1934.
29. Strain 161 isolated June 1935, from a sandwich. See J. Am. Med. Assoc. **105**, 1598, 1935.
30. Strain 166 isolated December 1936, from head cheese.
31. Strain 167 isolated April 1937, from sauce.

Mr. C. R. Tyler, New York City Department of Health

32. Strain Q 150 isolated from custard filling.

Dr. H. J. Shaughnessy, Illinois Department of Health, Springfield, Illinois

33. Strain Shrimp salad-2. No data received.
34. Strain Shrimp salad-3. No data received.
35. Strain Shrimp salad-1. No data received.
36. Strain Turkey 1 (S-10). No data received.
37. Strain Turkey 2 (S-11). No data received.
38. Strain Turkey 3 (S 12). No data received.
39. Strain Turkey 4 (S 13). No data received.
40. Strain Turkey 5 (S 14). No data received.
41. Strain Turkey 6 (S 15). No data received.
42. Strain Turkey B-6-A. No data received.

Mr. George J. Kupchik, New York University Medical School

43. Strain 32135 isolated in 1931 from "articles of food." Received from Dr. Ruth Gilbert, New York State Department of Health.
44. Strain 141 isolated from chocolate éclair by "Mr. Kremer of Milwaukee."
45. Strain S 2 Indianapolis isolated by Dr. G. M. Dack from tongue sandwiches.
46. Strain B 2 isolated by Drs. Shaughnessy and Grubb from milk.
47. Strain FP-18 (P-1) isolated from pie by Dr. C. E. Dolman.
48. Strain 9-303d isolated from chicken gravy by Drs. Jordan and McBroom (see Strain 9).
49. Strain 32132 isolated from canned crabmeat by Miss Marion B. Coleman of the New York State Health Department.
50. Strain 8-x-1 isolated from cake by Dr. G. M. Dack.

Dr. C. M. Carpenter, University of Rochester, Rochester, New York

51. Strain A isolated April 26, 1937, from chocolate éclair. See Health News (New York State Department of Health) **14**, 78, 1937.
52. Strain B isolated from French pastry. Same outbreak.
53. Strain C isolated from Kuchen. Same outbreak.
54. Strain D isolated from cream cake. Same outbreak.
55. Strain E isolated from chocolate cream pie. Same outbreak.

Mr. George J. Kupchik

56. Strain Mezo isolated from cow with mastitis by Drs. Shaughnessy and Grubb.

Information about the epidemiology and gastroenterotoxic properties of the strains was obtained from the donors.

The following strains from cows with mastitis were received from Dr. Wayne N. Plastridge, to whom the authors are indebted for data about the milk and cows: Strains S 346, S 440, S 905, S 375, S 899, S 393, S 347, and S 333.

TABLE 1

Results of Gastroenterotoxic and Stone Tests of Crude Cultures

	Animal tests reported		Stone tests on crude strains			
	Dolman	Other	Chapman	Stone	Kupchik [4]	Tanner
1			++++			
2	+ Kup.[1] (on S 70)		++++	Positive	Positive	
3	+ St.		++	++++		
4	+ Mey. (2), + Kup., + St. (2)		++++	++++	Positive	
5		0 C St.[2]	++++	Variable		
6	0 St. (2)	0 C St.		Variable	0	
7	+ St., + Kup.	+ C St.	++++	++++	0	
8		+ C St.	++++	Positive		
9			++++	Positive		
10			0	0		
11	0 St. 12/36, + Mey. 5/37		++++	++++		
12	0 Kup.		+++	Pos. (orig.)	0	
13	+ Kup.	+ K St. (2)	0	Positive [3]	0	
14						
15		+ H & K Tanner				Positive
16		+ K Tanner				Positive
17		+ H Tanner				0
18						0
19	+ Kup.				0	0
20						Positive
21	0 Kup.				0	Positive
22						0
23						0
24	+ Kup.				0	0

[1] Abbreviations: St.—Stone, Mey.—Meyer, Kup.—Kupchik, Tan.—Tanner, C—cat feeding, M—monkey feeding, K—kitten feeding, H—human volunteer, (2)—two tests were made. [2] Animal tests made on the éclair strains were positive for human and monkey (Meyer). [3] Stone reported a negative reaction 5/21/37. [4] Kupchik's results with medium C.

TABLE 1 (Concluded)

25						
26						
27						
28						
29						
30		+ M Dack 1/37				
31		+ M Dack /37				
32						
33						
34						
35						
36						
37						
38						
39						
40						
41						
42					0	
43	+ Kup.				0	
44	+ Kup.				Positive	
45	+ Kup.				0	
46	+ Kup.				0	
47	+ Kup.				Positive	
48	+ Kup.				Positive	
49	+ Kup.				0	
50	+ Kup.					
51						
52						
53						
54						
55						
56	+ Kup.				Positive	

The following strains, which were received along with some of the food-poisoning strains, were rejected for the following reasons:

23-99c	Enterococcus group.
Cherry-1	Degenerate gram-positive coccus (enterococcus?).
A-1	Stock *Staphylococcus albus.*
S-9 Milk	*Micrococcus tetragenus.*
Air-8	*Micrococcus tetragenus.*
Air-5	Isolated from air.
S-66	"Random" isolation from raw milk.
xS-19	"Random" isolation from raw milk.
S-15	Osteomyelitis.
S-88	Enterococcus? (Stone says that it "dissociated several times out of a regular type staphylococcus").
S-65	Abscess of elbow.
S-81	"Random" isolation from raw milk.
FP-10 (P-1)	Raw milk of apparently healthy cow.
137	Abscess of right thigh.
N3	Normal nasal mucous membrane.
FP-8 (E.A1)	Raw milk.
209	Stock *Staphylococcus aureus.*
16W	Wash water used for cleaning drinking glasses.
LV9	Vaginal discharge.

First studies were made on Dr. Stone's strains. It was not until after they had been completed that the significance of non-hemolytic variants was realized. In all subsequent cultures, variants with different hemolytic properties were separated. Sometimes a colony was selected as non-hemolytic but streaked transplants from it proved to be hemolytic. These variants are recorded as ⊙ and are considered evidence of degeneration of the strain.

Because of the importance of rabbit-blood agar for the detection on non-hemolytic variants, which were probably degenerate, the cultures were suspended in sterile water and diluted in such a way that a loopful, spread on a plate of rabbit-blood agar, gave discrete colonies. After 24 hours' incubation, colonies with different hemolytic properties were separated. The purified strains or variants were tested as follows:

Pigment: The culture was plated on proteose lactose agar or Loeffler's medium and incubated 48 hours. A loopful of the growth was collected and examined in a good light. Orange, lemon yellow, yellow, and light yellow growths were considered positive (+); while cream and white growths were considered negative (0). A color which could not be classified was designated as (±).

Hemolysis: A loopful of the culture was streaked on a plate of rabbit-blood agar and incubated overnight. Growths surrounded by wide, or moderately wide, hemolytic zones were considered positive; while those with slight hemolytic zones, or none at all, were considered negative.

Coagulase: A loopful of the culture from solid medium was mixed with .5 c.c. of fresh, oxalated human plasma and a second loopful was mixed with .5 c.c. of fresh rabbit plasma. After being shaken thoroughly, the tubes were placed in the incubator and examined at three hours and again overnight. If there was no apparent clot, the tubes were tilted to a horizontal position and examined for a jelly-like mass which could be seen rising slightly above the surface of the fluid or as an opaque disc. Any of these effects were considered positive. Unless otherwise noted, both plasmas gave similar results.

Crystal violet agar: A loopful of the culture from proteose lactose agar was streaked about two centimeters on a plate of crystal violet agar, described by Chapman and Berens (1935) and Chapman (1936), and incubated 36 hours. Orange and deep violet colored growths were considered positive; while white, pale violet, or mottled white and violet growths (sometimes with violet borders) were considered negative.

Bromthymol blue agar: A loopful of the culture was streaked over the surface of a plate of bromthymol blue agar, formula of Chapman, Lieb, Berens, and Curcio (1937), and incubated 36 to 48 hours. Strains that gave luxuriant growth were considered positive, while those producing poor or no growth were considered negative.

Mannitol fermentation: The culture was spread on a plate of Bacto phenol red mannitol agar and incubated overnight. Colonies surrounded by yellow zones were considered mannitol positive.

The results are reported in this order and are referred to as "P H C V B M" tests. In a few instances, lactose fermentation (L in Table 2) was also studied, using overnight incubation on Bacto phenol red lactose agar.

The criteria of Chapman, Berens, Peters, and Curcio (1934) were used in interpreting the results of pigment, hemolysis, and coagulase tests, which will be referred to as "PHC" tests. Any coagulating strain, also hemolytic aureus strains, were considered PHC positive. In most cases the PHC tests were parallel with other *in vitro* and *in vivo* tests.

According to this interpretation, 51 (91.1 per cent) of the 58 food-poisoning "strains" contained PHC positive variants. Assuming that the variant with the fullest complement of PHC properties more nearly represented the parent pathogenic strain, it will be referred

TABLE 2

Reactions of Dissociants of Food-Poisoning Cultures

	Tests made on major pathogenic variants					Minor pathogenic variants									
	Stone			No.	P H[1] C V B M L	Pct.	No.	P H C V B M	Stone 1	Stone 2	Stone 3	Pct.	No.	P H C V B M	Stone
	1st	2nd	3rd												
1	++++	++++	++++	63	+++++++		64	+⊙++++	++						
2	++++	++++	++++	149	++++++0										
3	+++	++++	++++	151	+++++++										
4	+	+++	++++	152	+++++++										
5	++++	++++	++++	153	±+++0++										
6	++++	++++	++++	154	±+++0++										
7	+++	++++	++++	155	+++++++										
8	0	+++	++	156	+++++++										
9	++++	++++	++++	157	+0 0+0 0 0										
10	0	+++	++	158	0++0+++										
11	++++	++++	++++	160	++++++0										
12	++++	++++	++++	162	0+0 0 0 0 0										
13	0	0	0	163	±0++++++										
14	++++	++++	++++	175	+++++++										
15	0	+++	+	187	+++++++										
16	++++	++++	++++	188	+++++++										
17	++++	++++	++++	189	++++++										
18	0	++	+++	190	++++++	30	197	+0++++	0	0	0				
19	++++	++++	++++	191	+++++++	10	198	+0++++	0	0	0	10	199	+0++++	0 0 0
20	++++	++++	++++	192	++++++										
21	++++	++++	++++	193	++++++	40	201	+0++++	0	0	0				
22	++++	++++	++++	202	+++++++	90	194	+0++++	0	0	0				
23	+++	++++	++++	203	++++++	90	195	+0++++	0	0	0				
24	+++	+++	++++	196	++++++										

[1] ⊙ Indicates that a non-hemolytic colony was selected but the transplant was hemolytic.

TABLE 2 (Concluded)

25.....	++++	++++	++++	204	++++++	20	245	+⊙++++	+++	·++++	++++				
26.....	0	+	0	205	++++++	10	246	0 0 0 0 0 0	0	0	0				
27.....	0	++++	++++	206	++++++										
28.....	++++	++++	++++	207	++++++										
29.....	+++	++++	++++	208	++++++	10	247	+⊙++++	+++	++++	++++				
30.....	+++	++++	++++	209	++++++	40	248	+⊙++++	++	++++	++++				
31.....	++++	++++	++++	210	++++++	20	249	+⊙++++	++++	++++	++++				
32.....	+++	+++	+++	211	+⊙++++										
33.....	0	++++	++++	216	± 0 0 0 0 0										
34.....	++++	++++	++++	217	++++++	10	250	+⊙++++	++++	++++	++++				
35.....	++++	++++	++++	218	++++++										
36.....	++++	++++	++++	219	++++++										
37.....	++++	++++	++++	252	++++++	40	251	+⊙++++	0	0	0	30	220	+++ 0 ++	++++ ++++ ++++
38.....	++++	++++	++++	221	++++++										
39.....	++++	++++	++++	222	++++++										
40.....	++++	++++	++++	223	++++++										
41.....	++++	++++	++++	224	++++++	20	253	+⊙++++	++++	++++	++++	10	254	+⊙++++	0 0 0
42.....	+++	++++	++++	225	+ 0 0 ++ 0										
43.....	0	0	0	228	+⊙++++										
44.....	++	+++	++	229	++++++										
45.....	++++	++++	++++	230	++++++	50	255	+ 0 ++++	0	0	0				
46.....	++++	++++	++++	265	++++++	80	231	+ 0 ++++	0	0	0				
47.....	+++	++++	++++	263	++++++	95	234	+ 0 ++++	0	0	0				
48.....	0	++++	+	236	++++++	5	261	0 0 0 0 0 0	+++	++++	++++				
49.....	++++	++++	++++	258	++++++	60	238	+⊙++++	+++	++++	++++				
50.....	0	+++	++	257	++++++	80	240	+⊙++++	0	0	0				
51.....	0	+++	++	266	++++++										
52.....	++	+++	++	267	++++++										
53.....	++	+	++	268	++++++										
54.....	0	+	++	269	++++++	30	271	+⊙++++	0	+	++				
55.....	0	++	++	270	++++++	20	272	+⊙++++	0	0	0				
56.....	0	0	0	233	0 0 0 0 ++										

to as the "major pathogenic variant." Some of the original cultures may not have represented the parent gastroenterotoxic strains, as the following analysis of the strains (if no variants were found) or their major pathogenic variants will show.

Forty-five "strains" or major pathogenic variants gave + + + + + + P H C V B M reactions.

Two strains (Q 150 and 32135) gave + ⊙ + + + + reactions.

Two strains (S 26-D and S 27) gave ± + + + 0 + reactions and were from the vomitus of a male and female victim respectively. Strains isolated from the éclairs causing the outbreak gave positive kitten and monkey feeding tests (Stone). Their properties may have been altered by the gastric juice because the Dolman test described by Dolman, Wilson, and Cockroft (1936) was negative on two kittens (Stone), cat feeding was negative (Stone), and the Stone reaction was variable (Stone) on one of the vomitus strains (S 27).

Strain S 53-4 gave 0 + + 0 + + reactions. This strain was sent by Dr. Stone and was marked "from a Stone negative colony." The Stone reaction was negative on repeated tests in our laboratory. The parent culture was Stone positive (Stone).

Strain S 10 gave ± 0 + + + + reactions and was isolated from raw milk. It is possible that this was a degenerate variant of the parent strain. On January 18, 1937, the Stone and Dolman tests were negative (Stone).

The following strains appear to have been degenerate forms or contaminants:

Strain S 51, giving + 0 0 + 0 0 reactions, was from a cow. Another culture (S 49) from the same cow had given + + + + + + reactions. Therefore, Strain S 51 was likely to be either a degenerate variant or a contaminant.

Strain S 3 gave 0 + 0 0 0 0 reactions. Kupchik (personal communication) expressed the opinion that this was a degenerate strain and, subsequently, Stone wrote that it had "dropped badly." The negative Dolman test (Kupchik) was later confirmed by Stone.

Strain Shrimp salad-2 gave ± 0 0 0 0 0 reactions. No data received.

Strain Turkey B-6-A gave + 0 0 + + 0 reactions. No data received.

Strain Mezo gave 0 0 0 0 + + reactions. It had been isolated from a cow with mastitis. In a number of strains from bovine mastitis the PHC tests were parallel with the clinical and analytical data (Table 3). Plastridge, Weirether, and Williams (1936) concluded that the hemolytic ability of mastitis strains was proportional to invasive power. The parallel between hemolytic activity and patho-

genicity is also found in staphylococci from other pathologic sources, according to Chapman, Berens, Nilson, and Curcio. Therefore, the conclusion seems justifiable that this strain was a degenerate variant or that it was a contaminant.

These results suggest strongly that food-poisoning staphylococci give + + + + + + P H C V B M reactions and that, when a culture reacts negatively to any or all of these reactions, it is probably a degenerate variant or a contaminant. Most pathogenic staphylococci, not associated with food-poisoning outbreaks, also give + + + + + + P H C V B M reactions, Chapman *et al.* (above). Therefore, a food-poisoning staphylococcus cannot be differentiated from pathogenic staphylococci by this combination of tests alone but, if a

TABLE 3

Tests of Staphylococci Isolated from Cows with Mastitis

Strain No.	Major dissociant P H C V B M L	Characteristics of milk		Stone reactions		
		Leucocytes [1]	Cl pct.[2]	1st	2nd	3rd
S 905[3].....	+++++++[4]	10,000,000	.....	++++	++++	++++
S 899[3].....	+++++++	2,000,000	.....	++++	++++	++++
S 393.....	+++++++[4]	1,900,000	.223	++++	++++	++++
S 347.....	+++++++	1,900,000	.176	+++	+++	+++
S 346.....	+0++++	3,000,000	.179	0	0	0
S 440.....	±0+00++	1,500,000	.22	0	0	0
S 375.....	++0++++	<100,000	.150	0	0	0
S 333.....	000++++	<100,000	.098	++++	++++	++++

[1] A leucocyte count in excess of 1,000,000 is generally regarded as suggestive of udder inflammation (Plastridge). [2] A chloride content of .14 per cent or less is frequently referred to in the literature as normal (Plastridge). [3] These two strains were from cows whose milk appeared abnormal and reacted positively to the bromthymol blue test of Plastridge. Other milk samples appeared normal and gave negative bromthymol blue tests (Plastridge). [4] Duplicate tests gave similar results.

strain or variant does not react positively to all six tests, it is unlikely to be the parent strain causing the food-poisoning outbreak.

These conclusions are in agreement with the statement of McBroom (1937) that "all hemolytic strains do not contain demonstrable enterotoxic properties for rhesus monkeys. All enterotoxic strains, conversely, appear to contain hemolysin." Kupchik expressed similar views.

CONSIDERATIONS OF THE STONE REACTION

Previous workers have failed to confirm the claim of Stone (1935) that food-poisoning strains can be differentiated from other staphylococci by their action on Stone's gelatin agar. The finding that many food-poisoning strains were mixtures of variants having different properties suggested the possibility that this might be responsible for errors in the Stone reaction. To test this possibility, food-poisoning

variants were tested for their Stone property. For comparison, tests were made on strains from bovine mastitis and from the noses and throats of chronic invalids. Bacto Stone's extract gelatin agar was used and tests were made on streaks (not spread) after 18 hours' incubation.

Stone positive strains were isolated from 32 (41.6 per cent) of 77 chronic invalids. Since only + + + and + + + + Stone reactions were recorded as positive, this would suggest that about one half of all chronic invalids harbor food-poisoning strains, which is unlikely.

TABLE 4

Stone Reactions of Staphylococci Isolated from Noses and Throats of Chronic Invalids

Method of isolation	P H C tests	Stone reaction	Number of strains	Pct. of total	Pct. Stone positive
Rabbit-blood	+	+	10	6.5	.....
agar	+	0	32	21.0	.....
	0	0	76	50.5	.....
	0	+	33	22.0	.....
		Total	151	100.0	28.6
Bromthymol	+	+	66	18.2	.....
blue agar	+	0	208	57.1	.....
	0	0	72	19.8	.....
	0	+	18	4.9	.....
		Total	364	100.0	23.1

Because food-poisoning staphylococci were shown to be of the + + + + + + P H C V B M type, 515 strains of staphylococci from chronic invalids were tested for their PHC and Stone properties. Only 6.5 per cent of 151 strains isolated from rabbit-blood agar were Stone positive and PHC positive, i.e., strains similar to most of the food-poisoning staphylococci (Table 4). Using the selective bromthymol blue agar for isolation, 18.2 per cent of 364 strains were Stone positive and PHC positive.

Of the PHC negative group from rabbit-blood agar, i.e., strains not likely to be food-poisoning staphylococci, 22 per cent were Stone positive. To determine their possible significance Dr. Stone had Dolman tests made on a few of these strains, selected because of their different PHC-Stone combinations. Although the number of strains tested was small, the results (Table 5) suggest that the Stone reaction of PHC negative strains has no significance.

Since non-hemolytic variants are likely to be degenerate, the proportion of positive Stone reactions should be proportionate to the hemolytic properties of the variants. Consistently positive Stone reactions were obtained in 70.5 per cent of hemolytic, 57.1 per cent of variable hemolytic, and 0 per cent of non-hemolytic variants (Table 6). This suggested either that the Stone factor was associated with hemolysin or that it degenerated along with hemolysin. That it was not identical with hemolysin is shown by the fact that it was positive in only 32 (27.6 per cent) of 116 strains which gave + + + + + + P H C V B M reactions but which were not connected with food-poisoning outbreaks.

These experiments indicate that the Stone reaction is positive in at least two-thirds of major pathogenic variants of food-poisoning

TABLE 5

Comparison of In Vitro, Stone, and Dolman Tests of Staphylococci

Strain No.	*In vitro* tests P H C V B M	Stone reactions made by		Dolman tests
		Stone	Chapman	
4893	0 0 0 0 0 0	++++	Positive	0
4895	0 0 0 0 0 0	0	0	0
4899	0 0 0 0 0 0	0	0	0
4898	++++++	++	0	0
4900	++++++	++++, later 0	0	0
4894	++++++	0, later ++++	Positive	Positive on repeated tests

strains and that degeneration of the strain, as indicated by loss of hemolytic property, usually is accompanied by loss of the Stone factor. These facts, together with the disproportion between the incidence of Stone reactions in + + + + + + P H C V B M food-poisoning and non-food-poisoning strains, are strongly suggestive that the Stone reaction is associated with the gastroenterotoxic property of staphylococci.

Further proof that the Stone factor is independent of general pathogenicity of staphylococci is shown by the fact that in non-food-poisoning staphylococci the proportion of Stone positive strains in PHC positive and negative groups was similar (26.2 and 30.3 per cent, respectively).

Irregularities in results obtained with the Stone reaction can be traced to at least four factors:

1. The reaction is applied to strains which do not give + + + + + + P H C V B M reactions, i.e., strains which may not be typical food-poisoning staphylococci. The negative results reported by Tanner for Strains R-1 and R-2 and by Kupchik for Strains B-2 and FP-18

can be explained by the fact that these strains contained between 80 and 95 per cent of non-hemolytic, Stone negative variants. In these four instances the crude cultures had been reported by them as Stone negative, while the + + + + + + P H C V B M variants from the crude cultures were Stone positive. This indicates that some of the confusion in interpreting the Stone reaction can be attributed to the improper selection of "strains" or the use of mixed dissociants.

2. In other instances the different results obtained in different laboratories might be explained by the possibility that the composi-

TABLE 6

Relation Between Stone Reaction and Degeneration of Staphylococci

	P H C V B M	
Food-poisoning strains	+ + + + + +	31 of 44 were consistently Stone positive (70.5%[1]).
	+ ⊙ + + + +	8 of 14 were consistently Stone positive (57.1%).
	+ 0 + + + +	0 of 9 were consistently Stone positive (0%).
Non-food-poisoning strains	+ + + + + +	32 of 116 were consistently Stone positive (27.6%).

Reactions of Degenerate Variants not Included in Above

Variant No.	Strain No.	*In vitro* tests P H C V B M	Stone test
153	S 26-D	± + + + 0 +	+
154	S 27	± + + + 0 +	+
220	Turkey-2	± + + 0 + +	+
163	S 10	± 0 + + + +	0
157	S 51	+ 0 0 + 0 0	+
162	S 3	0 + 0 0 0 0	+
225	Turkey B-6-A	+ 0 0 + + 0	+
261	9	0 0 0 0 0 0	+
158	S 53-4	0 + + 0 + +	0
216	Shrimp salad-2	± 0 0 0 0 0	0
233	Mezo	0 0 0 0 + +	0
246	9	0 0 0 0 0 0	0

[1] When the Stone reaction was not +++ or ++++ on three occasions, it was considered negative. However, this eliminates Strains 152 and 206 which gave +++ or ++++ reactions on two out of three tests but were not considered positive. If these two strains are included with the positive group, then the percentage of Stone positive strains in this group becomes 75.

tion of the Stone medium varied in some essential factor. Kupchik reported that, using four different "Stone media," widely different results were obtained. Stone (1935) found that a number of ingredients of culture media affected the Stone reaction.

3. If Stone reactions of + + + + and + + + are recorded as positive, while reactions of + +, +, and 0 are considered negative, a strain giving intermediate reactions (+ + + or + +) would be

considered positive when the reaction was + + + but negative when it was + +.

4. Variations noted when a strain was retested for its Stone property suggested that the strain might have been in different "phases," to borrow a term from the Salmonella group. To test this possibility, a number of strains were tested at three different times. (There was no significant difference between tests made after 24 and 48 hours' incubation.) The results (Tables 1, 2, and 7) show that while most strains gave constant results (87.6 per cent), some of them varied considerably. A parallel appears to occur in the hemolytic properties of staphylococci. Chapman *et al.* (1937) showed that, when strains were

TABLE 7

Differences Observed When the Stone Reaction of Strains Was Tested at Three Different Times

Strains showing no essential difference				Strains showing significant differences			
++++	++++	++++	37	++++	++++	++	4
++++	++++	+++	12	++++	+++	+	1
++++	+++	+++	1	++++	+++	0	2
+++	+++	+++	2	++++	+	0	1
+++	++	++	2	+++	++	0	5
+++	++	+	1	+++	+	0	1
++	++	+	3				—
++	++	0	1	Total			14 (12.4%)
++	+	+	4				
++	+	0	4				
++	0	0	1				
+	0	0	5				
0	0	0	26				
			—				
Total			99 (87.6%)				

retested after storage for one month, major differences in hemolytic power occurred in seven per cent of the strains. This fluctuation of the hemolytic activity had been noted by others and may be explained by fluctuations in the proportion of different components. Stone (personal communication) claims to have worked out a method for "stabilizing" the cultural reactions of certain strains, but details are not available at the time of writing.

In a study of the hemolytic and Stone properties of food-poisoning strains, the proportion of positive, variable, and negative strains was similar in both tests (Table 8). This indicates that the tendency of strains to lose their Stone property is similar to their tendency to lose their hemolytic property.

Although some strains showed considerable degeneration, as determined by the proportion of degenerate variants, there was no constant relation to the age of the strains. Strains 95 and McB/3, isolated in 1932, showed no evidence of deterioration. Strain 1, isolated in 1929, gave strong Stone reactions and contained only 20 per cent of a variant giving variable hemolysis. On the other hand, Strains c-2, c-3, c-6, R-1, and R-2, isolated from one outbreak, showed considerable degeneration of P H C V B M properties, although the Stone factor was consistently positive.

The fact that strains from the same source showed similar degeneration indicates that the tendency to degenerate differs with each strain and is characteristic for that strain. Strain 9 contained 10 per cent of a variant which gave 0 0 0 0 0 0 P H C V B M reactions. Strain 9-303d, which may have been identical with it, also contained

TABLE 8

Degeneration of Food-Poisoning Staphylococci as Shown by Loss of Hemolytic and Stone Properties

	Hemolysin		Stone	
	Number	Pct.	Number	Pct.
Positive	18	45.0	10	43.4
Variable	13	32.5	7	30.4
Negative	9	22.5	6	26.2
Total	40	100.0	23	100.0

five per cent of a similar variant. This would suggest that the type of degeneration is constant also. Curiously, one of the 0 0 0 0 0 0 variants (No. 261) gave positive Stone reactions, while the other 0 0 0 0 0 0 variant and the major pathogenic variants from these two strains were, with one exception, Stone negative.

Strains A, B, C, D, and E gave weak or negative Stone reactions. The two latter showed some evidence of degeneration but none could be detected in the other three. All were from the same outbreak but from different sources. It is possible that the parent strain contained very little Stone factor but, since nearly all recent strains have given positive Stone reactions, it is more likely that the Stone factor was unusually labile in this group of cultures. Degeneration usually is accompanied by loss of the Stone factor, but Variant 261, for example, showed degeneration of the P H C V B M properties although repeated Stone tests were + + + +.

Although the Stone reaction was positive in 30.3 per cent of nonpathogenic, non-food-poisoning strains, Kupchik reported the Dolman

test positive in nine (47.3 per cent) of 19 non-food-poisoning strains (Table 9). The Dolman test was negative in 20 per cent of Kupchik's food-poisoning strains.

These considerations favor the view that the Stone reaction is associated with the gastroenterotoxic property of staphylococci although it may not be identical with it. The association of gastroenterotoxic properties and general pathogenic properties in food-poisoning staphylococci and the separation of these two properties in non-food-poisoning strains suggests that a combination of the two properties may be necessary for a strain to produce food-poisoning outbreaks. This would explain McBroom's observation and might point the way to an improved animal-inoculation test for gastroenterotoxic strains.

The results obtained in this investigation point to two possible sources of food-poisoning outbreaks. The frequency with which cows

TABLE 9

Comparison of Dolman Tests with Food-Poisoning Histories of Staphylococci[1]

Food-poisoning history	Dolman test	Number of strains
+	+	20
+	0	5
0	+	9
0	0	10
		—
		Total 44

[1] Rearranged from Kupchik's data.

and milk were suspected and the fact that most strains from staphylococcic bovine mastitis were Stone positive (Table 3) suggest that the cow may be an important factor. However, the possibility that the infection may originate in food handlers cannot be ignored. It will be shown by Chapman *et al.* (4th reference) that 427 (24.9 per cent) of 1,713 strains from chronic invalids (persons with sinusitis, headaches, arthritis, eye diseases, and minor ailments) gave + + + + P H C V reactions. If only 26.2 per cent of PHC positive strains are Stone positive (Table 4), then only 6.52 per cent of staphylococci from chronic invalids are likely to be food-poisoning strains. This suggests that prevention of epidemics may depend upon control of cows and routine study of food handlers.

From a summary of these facts it is possible to suggest methods for the isolation and preliminary differentiation of probable food-poisoning staphylococci. With the exception of Strains S 51, S 3, and Shrimp salad-2, which were probably degenerate representatives of the parent strains, all the major pathogenic variants or strains of

food-poisoning staphylococci would have been isolated if the cultures had been treated by the method of Chapman, Lieb, and Curcio. Therefore, the following outline is suggested for the isolation of food-poisoning staphylococci:

Plate the suspected material on (1) Stone's extract gelatin agar to detect Stone positive colonies; (2) rabbit-blood agar to detect hemolytic colonies; (3) phenol red mannitol agar to detect mannitol-fermenting colonies; and (4) bromthymol blue agar to detect probable pathogenic colonies. Positive colonies should be confirmed by the coagulase test. A culture which gives positive results on all four media and coagulates plasma is probably a food-poisoning staphylococcus. Further tests may then be made to confirm the diagnosis.

These results suggest that further study of the Stone reaction should be made to increase its stability, sensitivity, and specificity.

SUMMARY AND CONCLUSIONS

1. Typical food-poisoning staphylococci, in common with other pathogenic staphylococci, produce yellow or orange pigment, hemolyze rabbit-blood agar, coagulate human and rabbit plasma, produce orange or deep violet growths on crystal violet agar, grow luxuriantly on bromthymol blue agar, and ferment mannitol.

2. Loss of any of these properties indicates degeneration of the strain.

3. Hemolysis appears to be the best indicator of degeneration.

4. The Stone reaction was positive in 70.5 per cent of typical food-poisoning staphylococci.

5. The Stone reaction was positive in 27.6 per cent of strains with similar properties but isolated from non-food-poisoning sources.

6. The Stone reaction was positive in 57.1 per cent of food-poisoning strains which showed partial degeneration of the hemolytic property.

7. The Stone reaction was negative in all 10 non-hemolytic food-poisoning strains or variants which reacted positively to the other tests mentioned above.

8. This suggests that the Stone reaction should be applied only to variants which react positively to all the tests mentioned. Degenerate strains may be lacking in the Stone property.

9. Errors in interpreting the Stone reaction may be explained by the application of the reaction to crude strains which may contain degenerate variants, differences in composition of the Stone medium, changes in the "phase" of the culture, and the difficulty of interpreting intermediate reactions.

10. The tendency to degenerate is characteristic for each strain and is not related to age of the culture.

11. Food-poisoning staphylococci appear to represent a combination of the gastroenterotoxic factor with the general pathogenic property of staphylococci.

12. Food-poisoning staphylococci may be isolated by plating the suspected material on Stone's extract gelatin agar, rabbit-blood agar, phenol red mannitol agar, and bromthymol blue agar and comparing the results. A typical food-poisoning staphylococcus will react positively on all four media and will coagulate human and rabbit plasma.

13. It is suggested that further study of the Stone reaction be made on separated variants.

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APPARATUS FOR DETERMINATION OF SPORE-DESTRUCTION RATES

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(Received for publication, June 30, 1937)

In recent years it has been customary to use the method of Esty and Williams (1924) in the determination of heat-resistance values for spores. These values have been used in the calculation of processes for canned foods. By this method attempts are made to measure the absolute death time of the suspension and, except in cases of special study, there has been no attempt to measure the course of destruction.

Viljoen (1926) demonstrated that when a suspension of resistant spores was heated at 110°C.(230°F.) there was a logarithmic decline in numbers of survivors. This, together with other reports, points unmistakably to the fact of material variation in resistance of individual spores making up a spore suspension. On this basis it has been assumed that one principal cause of failure to obtain concordant results in successive heat-resistance runs is the inevitable variation in the resistance of the spores making up the small group which persisted to the end of the heating time.

Levine, Buchanan, and Lease (1927), in studying the relative germicidal efficiencies of strong alkalis, have also remarked upon variation in the resistance of organisms in a suspension and based their study upon the determination of destruction rates. In this report reference was made to the error arising through low counts occurring toward the end of the test, and the killing time of 99.9 per cent of the exposed bacteria was taken as the end-point and was regarded as a suitable criterion of the relative germicidal efficiencies of the alkali solutions which were studied.

After studying the reports referred to, consideration was given to the matter of modification of the Esty and Williams' method to yield information on destruction rates rather than absolute death times. By making interval counts from thermal death-time tubes, as was done by Viljoen, it was possible to obtain such data; but the method, as applied in this manner, was cumbersome and there was some error arising from failure to completely culture the tube contents. Therefore, from the standpoint of convenience of method as well as more accurate procedure, experimental work was begun in order to find a method which would make possible the heating of a mass suspension

under pressure and the withdrawal of test samples without disturbing the conditions of the heating test. Levine used a properly equipped Woulff bottle in his tests, which were conducted under atmospheric pressure. Obviously such equipment could not be used for similar tests where the spores were of high resistance to heat and where it was necessary to apply temperatures well above boiling. Equipment satisfactory for the purpose desired must satisfy the requirements that (a) it could be heated under pressure with close

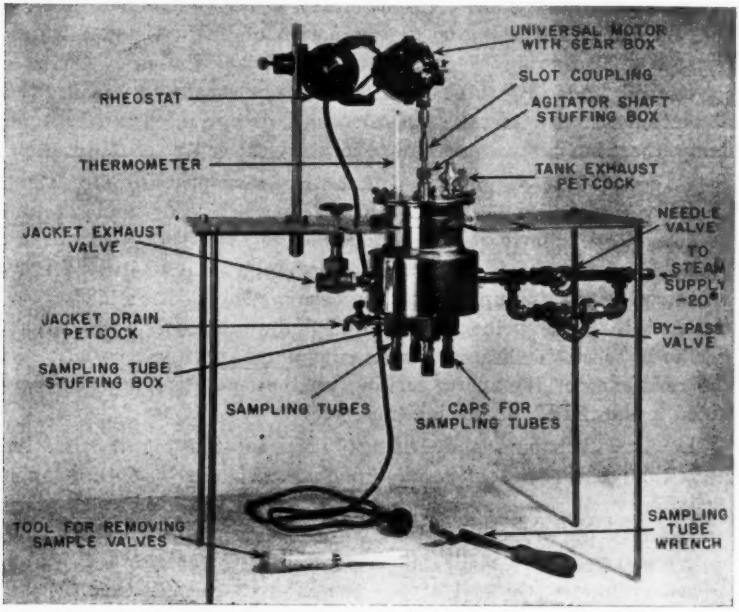

FIG. 1. Photograph of heat-resistance apparatus.

control of temperature, (b) samples could be withdrawn aseptically without disturbing the test conditions, and (c) there should be no mechanical limitation as to the number of samples which could be taken during the test.

DESCRIPTION OF APPARATUS

The equipment described here was designed and constructed to meet the requirements specified above. In general it consists of a cylinder 4 inches in diameter and 4½ inches deep, with a steam jacket ¾ of an inch wide covering the bottom and extending up the sides for about 2 inches (Figs. 1 and 2). Construction of the tank

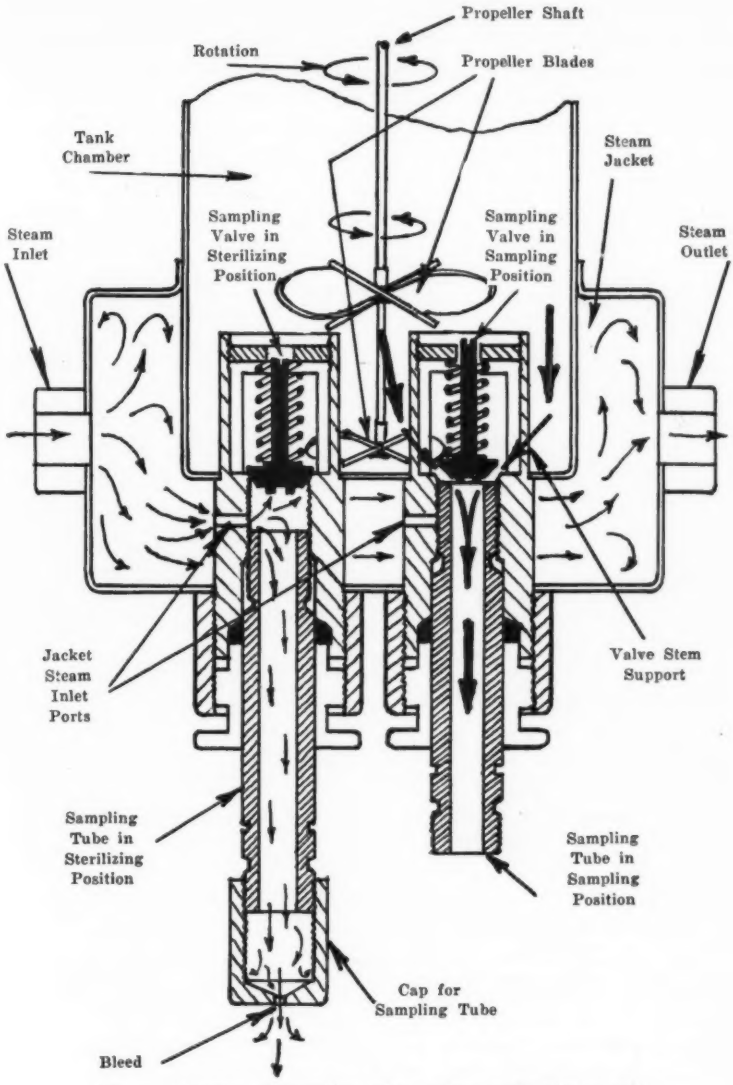

FIG. 2. Diagram of sampling tubes, valves, and agitator unit.

chamber and steam jacket is of stainless steel tubing with a wall thickness of 1/8 of an inch. The capacity of the chamber is approximately 900 c.c. Through the bottom are installed four sampling tubes which are connected with sampling valves at the bottom of the tank. The cover, also of 1/8-inch stainless steel, is fitted with a 1/8-inch tank exhaust petcock, a flange fitting for a rubber stopper to hold the thermometer, and a stuffing box for the agitator shaft. The cover is held in place by eight studs equipped with wing nuts. A copper gasket is used between the cover and the tank in order to obtain a tight seal. The jacket is fitted with two taps for 1/4-inch standard pipe for steam supply and exhaust, and with a jacket drain petcock at the bottom.

The agitator unit consists of two four-bladed propellers mounted on a single shaft. The propellers are arranged to produce opposing currents and, thereby, vigorous agitation. The bottom propeller is one inch in diameter and draws the liquid in an upward direction, while the top propeller is two inches in diameter and opposes the action of the former. Both propellers are located on the shaft below the level of the top of the steam jacket.

A special problem in design was presented by the sampling requirement and among the most important features of this apparatus are the four sampling valves and tubes. The valves, constructed of stainless steel with faces at angles of 75 degrees with the horizontal, are mounted in the bottom of the tank proper. The valve faces are ground into seats with the tops of the valves about flush with the bottom of the tank. The valve stems project up into the tank and are held in place by valve cages so that the liquid may circulate freely across the sampling valves. Tension is supplied to the valves by means of nine-turn silver-plated springs. The valve stem supports act as guides for the vertical movement of the valves. The bottom faces of the valves are fitted with three small lugs which engage the top of the sampling tubes, thus providing openings for the liquid to flow from the tank through the valves and into the tubes.

The sampling devices are stainless steel tubes 3 1/8 inches long with holes 1/4 of an inch in diameter through the entire length. They are threaded to fit parts which go through the jacket at the bottom integral with the valve stem supports mentioned above. These parts have steam inlets into the jacket which utilize the jacket steam pressure for sterilizing the sampling tubes, valve faces, etc. This procedure will be described later. The bottoms of the sampling tubes are fitted with screw caps into which are drilled holes 1/64 of an inch in diameter, for bleeding. The tubes have slots to receive a special swivel wrench for adjusting to the various positions of sampling,

sterilizing, etc. Acknowledgment is made to the American Instrument Company, Silver Spring, Maryland, the manufacturers of the apparatus, for the many valuable suggestions made regarding problems arising during fabrication.

METHOD OF OPERATION

In describing the sampling and sterilizing procedure it is appropriate to cover a complete cycle in order that the functions of the various units may be more readily explained. At the start of the test all of the sampling assemblies are sterile and the sampling tubes are in the sterilizing position (Fig. 2); that is, the tops of the sampling tubes are below the jacket steam-inlet ports which allow saturated steam under the jacket pressure to be in contact with the bottoms of the valves, the sample tube threads, and the entire bores of the sampling tubes. Bleeding occurs through the $\frac{1}{64}$-inch holes in the caps described above. About 30 seconds prior to sampling time the tube to be used is screwed up with the wrench to the point where the top of the tube just touches the lugs on the bottom of the sample valve. This operation closes the jacket steam inlet to the sampling tube and while in this position the cap may be removed since it is no longer under pressure. At the actual sampling time the tube is advanced further and this raises the valve and allows the liquid within the tank to be expelled, under pressure, through the tube. Five to 10 c.c. are allowed to run through the tube before the sample for culturing (about 10 c.c.) is taken in a sterile collecting tube. Following this operation the sample tube is backed off until the valve closes.

Before again being placed in the position where it is to be sterilized, the tube is screwed down to a point where the top is just above the jacket inlet. There is no flow of steam through the tube at this point and it is thoroughly flushed with sterile water. This is done by means of a wash bottle fitted with a special small-bore copper nozzle. After being flushed with water the cap is replaced and the tube is lowered to the original point where the steam under jacket pressure flows through the entire sampling assembly, external to the chamber.

In order that the temperature within the apparatus may be closely controlled, it is connected by rubber pressure hose to the exhaust valve of a large autoclave which is kept constantly at about 20 pounds pressure. The flow of steam into the jacket is directly controlled by means of a needle valve. When this valve is once set for a given temperature it holds within very close limits. Normal variation is .2°C.±. The needle valve is by-passed by a standard globe

valve in order that the apparatus may be brought to test temperature as rapidly as possible.

Following collection of the samples, they are cultured in appropriate media and incubated under proper conditions. Resistant "flat-sour" thermophilic spores are cultured in tryptone agar and are incubated at 55°C.(131°F.). Putrefactive anaerobes are cultured in liver agar, or other suitable protein medium, and plates are incubated at 37°C.(98.6°F.) in anaerobic jars.

TABLE 1

Spore Destruction Rates of Organism No. 1518 (3-8-35) in Phosphate pH 6.95 at Various Temperatures

110°C.(230°F.)		115°C.(239°F.)		120°C.(248°F.)	
Time	Surviving spores in 1 c.c.	Time	Surviving spores in 1 c.c.	Time	Surviving spores in 1 c.c.
min.		*min.*		*min.*	
0	60,000	0	50,000	0	50,000
25	15,000	5	30,000	5	11,000
50	8,000	10	19,000	7	7,500
75	4,500	15	15,000	10	2,800
100	2,500	20	13,000	12	1,600
125	1,040	30	8,500	15	600
150	470	40	3,300	17	250
175	160	50	1,900	20	135
200	53	60	760	22	48
225	27	70	400	25	15
250	7	75	215	27	8
275	2	80	84	30	2
300	5 in 6 c.c.	85	35	32	1
325	0	90	28	35	2 in 6 c.c.
350	0	95	14	37	0
375	0	100	4	40	0
400	0	110	2 in 6 c.c.	50	0
.....	.....	120	0	60	0
.....	.....	130	0	.....	.....

TYPICAL RESULTS

Typical data are presented to illustrate heat-resistance runs of organism No. 1518, a facultative thermophilic "flat-sour" organism of much higher than usual resistance to heat (Table 1 and Fig. 3).

SUMMARY

New equipment for use in the determination of spore-destruction rates and the method of its operation are described. The principle of the equipment was suggested by the necessity for obtaining death-

time data relating to decrease in numbers of spores under the influence of heat, rather than absolute death times. Typical data illustrate results obtained by this method.

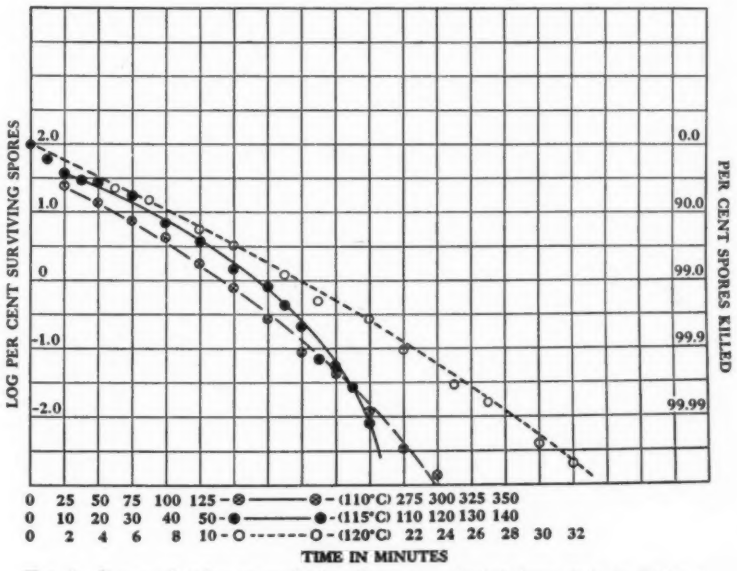

FIG. 3. Curves showing spore-destruction rates plotted from data in Table 1.

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POTATO MEALINESS AND CHANGES IN SOFTNESS ON COOKING

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(Received for publication, July 2, 1937)

Many publications [Coudon and Bussard (1897), Gilmore (1905), Harcourt (1907), East (1908), Langworthy (1917), Goldthwaite (1925), Cobb (1935), and Sweetman (1936)] on the subject of potato mealiness admit a relationship between the mealiness, or texture, of

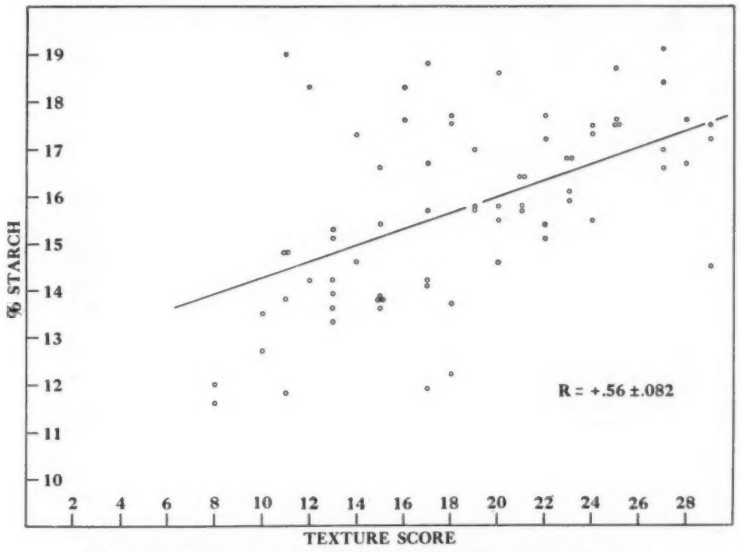

FIG. 1. Influence of starch content of potatoes on texture scores.

cooked potatoes and the starch content. The correlation coefficients reported range from +.25 to +.62. The most accurate results of this laboratory (Fig. 1) yield a correlation coefficient of +.56 ±.082 between starch and texture. These data include six varieties grown at twelve locations throughout the state of Colorado during the summer of 1936 on dryland and on irrigated farms.

Investigators have attempted to correlate protein content, or a starch-protein ratio, to texture. Coudon and Bussard, Langworthy,

and Cobb claimed to have found some relationships; East, Harcourt, and Butler, Morrison and Boll (1913) found none.

The correlation coefficient between nitrogen and texture was found to be —.34 ±.104 for the samples grown under conditions in Colorado. This is a barely significant value. Yet, on working out a multiple correlation between texture and the two components, nitrogen and starch, or a simple correlation between texture and the starch-nitrogen ratio, a coefficient of +.65 was obtained. This is slightly greater than the +.56 that was obtained for starch and texture alone, but the difference is not significant statistically, which means that it is still uncertain whether protein nitrogen really contributes to mealiness.

From these studies there is apparently no doubt that the starch content is the major factor contributing to mealiness. However, the question this laboratory would like to answer is, "What other factor or factors contribute to the texture scores and account for the difference between .65 and 1.00 in the correlation coefficient?" Attempts have been made to correlate all the remaining major constituents of the potato, such as water, fat and fiber, and ash and ash constituents; but so far nothing significantly correlated with the texture score has been found except starch, nitrogen, and water. The chemical analyses available, which were carried out by the chemistry section of the Colorado Station, contained only water, starch, total nitrogen, and ash and ash constituents, the remaining material being designated as fat and fiber. No attempt was made to analyze for pectins, hemicellulose, cellulose, etc.

It is generally agreed, as shown by Sweetman (1936), that the disintegration of cooked potatoes described by the term "mealiness" is a separation of the plant cells in the tuber and not a breaking of these cells releasing starch grains. Sweetman (1933, 1936) states there is evidence that these cells are held together by material known as protopectin. Therefore, cooked potatoes were analyzed for protopectin by a method used by Dastur and Agnihotri (1934). Since starch was known to be a factor contributing to mealiness, it was eliminated from consideration by choosing samples that had approximately equal starch content but differing degrees of mealiness. From the samples available it was possible to choose four pairs which differed from each other only in location grown and in texture score. The analysis of pectins, although not giving satisfactory checks in most cases, gave no indication that more protopectin was present in the less mealy tubers.

Microscopic examination of the cell size of these eight samples of cooked tubers gave no consistent results. In three cases the more

mealy of the pairs had the largest cells, but in the fourth pair the most mealy had the smallest cells.

One time-tested method of determining whether or not potatoes are cooked done is to pierce them with some pointed instrument to gauge the softness. From this grew the idea that a penetrometer could be used to test the degree of doneness of potatoes, and it was introduced and used by Landreth (1929), Sweetman and Lancaster (1931, 1932), and Thiessen (1934). Since in these experiments it was desired to measure the characteristics of the tuber as a unit, and since Sweetman and Lancaster reported differences in the rates of cooking of the bud and stem ends, and Thiessen (1935) found differ-

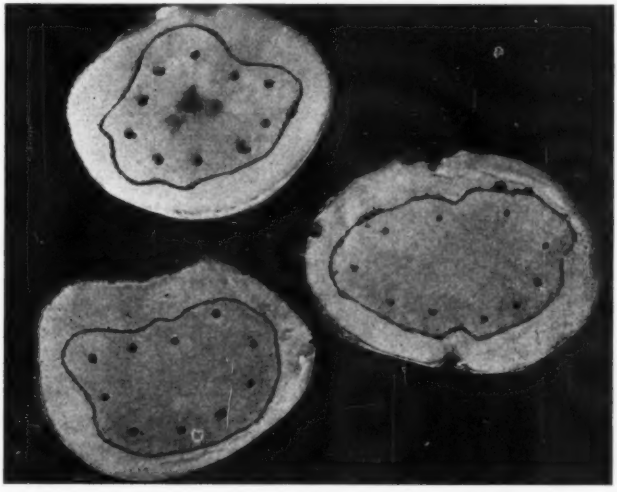

FIG. 2. The line traces the cortical ring of the potato slices and the dots illustrate the points at which the penetration measurements were made.

ences between the rates of cooking of the cortex and medullary areas, it was decided to make the penetration measurements on the outer medullary area. A slice two centimeters thick was cut from the center of each tuber in a plane perpendicular to a line joining the bud and stem ends. Each slice was cooked individually for 25 minutes in boiling water at 95°C.(203°F.). The black line in the illustration outlines the cortical ring, and the dots indicate the points of the ten measurements of penetration made on each slice (Fig. 2). These ten tests were made on 18 or more tubers and averaged.

On plotting the results obtained during the past three years against the starch content of the whole tuber, a marked relationship

was noted between the penetration and the starch content, yielding a correlation coefficient of —.70 ±.080. In other words, the higher the starch content the lower the penetration, or the less the softening on cooking (Fig. 3). Going back to Sweetman's and Thiessen's work on rate of softening of different areas of the tuber and to Glynne and Jackson's (1919) work on starch content of these same regions it was found that those sections which softened quickest also had the lowest starch content.

These findings agree with the work of Rathsack (1935) of Germany who used the rate of cutting by a fine wire acting under a

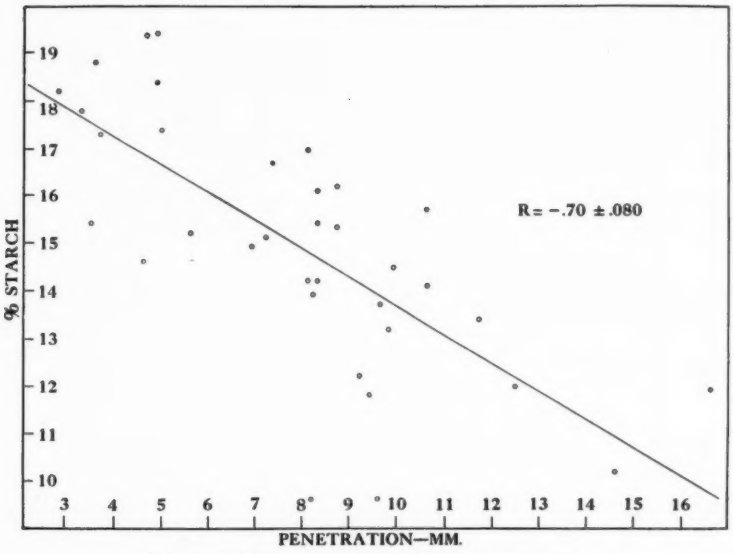

FIG. 3. Relation of starch content to penetration.

definite weight. He found a correlation coefficient of +.53 between the "schnittfestigkeit" and the starch content.

Regardless of the relatively wide range of degree of penetration or softness of potatoes, the degree of "doneness" to taste does not seem to be related to softness. Evidently the cooking of a potato so that it will taste done is more dependent on the time than on softness. When cooked done a potato has a mellow feel to the tongue, while in the raw state it is slightly crisp in addition to having a distinct difference in flavor.

Since there is such a definite relationship between texture and starch and between penetration and starch, one might expect a high

correlation between texture and penetration. Unfortunately, this was not the case.

Since the outside of a tuber heats up first during cooking, it actually cooks for a longer period than the center portion. It is therefore fortunate that the center area contains the least starch. This allows the center to cook to the same degree of softness in about the same time as the outside portions, although it is not maintained at a high temperature for so long a time. It also may be pointed out that potatoes which fail to cook uniformly may have an improper distribution of starch.

During the past year penetration tests were carried out on samples of five varieties of potatoes cooked for varying lengths of time. In obtaining the samples, however, two separate samples of the same variety were received in two cases—Bliss Triumph and Katahdin.

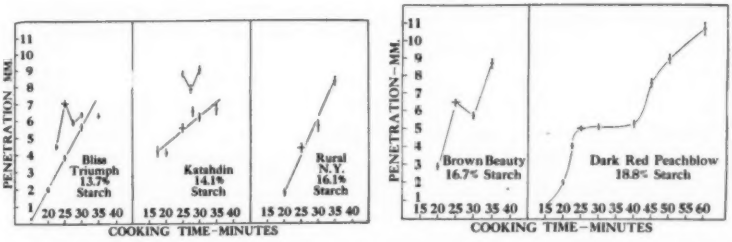

FIG. 4. Relation of penetration to cooking time for five varieties of potatoes grown at the same location.

Needles of various sizes were used for testing the different samples so as to make the depth of penetration small enough to cover the whole cooking range without changing conditions of the experiment in any way except the cooking time. The results of this study show how the tuber softens as cooking progresses (Fig. 4). The circles represent average values and the arrows show the standard deviation of these mean values. The circles with the horizontal line represent the points at which the tubers were cooked "done" as judged by taste.

These experiments were carried out in March and April, hence it was possible to cook the samples for as long as 60 minutes without disintegration. The reason this can be done in the spring and not in the fall has been shown by Rathsack (1935).

The graphs of penetration versus cooking time show that the softening is progressive until the tuber is done or has been cooked about 25 minutes. In cases where small enough increments of cooking time were taken, a slight hardening seemed to take place on further cooking, surprising as it may seem. While the differences between the

values do not show a statistically significant decrease in every case, they do show that the softening which has been previously taking place does not continue but is arrested. In the last case, Dark Red Peachblow, there is certainly no doubt about this effect being real. This phenomenon has also been pointed out by Rathsack by means of two entirely different methods.

Just what this means and what it contributes to our knowledge of the cooking tuber is doubtful. At least it can be said that cooking is not a mere gelatinization of the starch, since the softening is not progressive. There are evidently two changes taking place, the first of which goes to completion and is finished when the tuber is done, the second of which continues more slowly and finally results in a greater softness than the first. Further than that, there seems to be nothing of value at present.

SUMMARY

Another group of data has been added to the literature on correlation of texture and starch. It is realized, however, that starch is only one of the factors contributing to a desirable texture. Protein content seems to have some influence. A high relationship has been established between starch content and softening as measured by the penetrometer. It is also shown that there is a plateau in the softening of potatoes during cooking and perhaps an actual hardening at a certain stage. While this study has not yielded much of value to a solution of the factors affecting texture, it is by means of some such attack that progress may be gained along this line.

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A COMPARISON OF VARIOUS TREATMENTS OF CEMENT AND STEEL WINE-TANK SURFACES [1, 2]

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(Received for publication, July 20, 1937)

In larger wineries of California, as well as in certain other wine regions, particularly French North Africa, Australia, and Argentina, cement tanks are used rather commonly for fermentation of must and storage of wines. Many of these storage tanks are of large capacity, 30,000 to 50,000 gallons each; one in California holds approximately 250,000 gallons. At present, common wines are shipped from California in steel tank cars lined with various coatings. Owing to the fact that there is considerable reaction between concrete or steel and wine, there is need for information on the comparative value of various treatments and coatings for inside surfaces of concrete tanks and steel tank cars.

Fabre (1920) states that in French North Africa concrete tanks made with some cements impart considerable iron to wine and, therefore, recommends that cements be selected which are low in iron compounds soluble in wine. He states also that wine stored in new concrete tanks, in which the inside surfaces have not been "delimed" or coated, dissolves an excessive amount of lime with resulting adverse effect on the quality of the wine. He recommends coating the interior with a sand-cement mixture that is very rich in cement. When this coating has hardened, he recommends that the tank be "delimed" by filling it with cold water which is left for several days, draining out the water, washing the surface with 20-per cent tartaric acid solution, allowing this wash to dry, repeating the washing with the acid, and allowing that to dry. He states that potassium silicate and sodium silicate solutions, hydrofluosilicic acid, paraffin, and various other coatings have been used but that in his estimation the simple "deliming" treatment described above is the most satisfactory.

H. E. Laffer (unpublished oral report at Third Annual Wine Conference, Berkeley, 1936) stated that in Australian wineries the inside surface of new concrete tanks is finished as smoothly as possible with a rich sand and cement mixture, and after hardening of this surface

[1] From the Fruit Products Laboratory, University of California.

[2] The writers desire to thank Professor Troxwell and E. Brown of the Civil Engineering Department, University of California, for very valuable advice and assistance and for use of the Department's facilities in preparing the concrete cylinders used in the tests.

the tank is "soaked out" with water. It is then treated with one or more successive washes of 25-per cent tartaric acid solution to form a coat of insoluble calcium tartrate. The surface is allowed to dry and is then coated heavily with melted paraffin. Wine stored for long periods deposits on the walls of the tanks an encrustation of argols—nearly pure cream of tartar, $KH(C_4H_4O_6)$, a valuable by-product. This is recovered by scraping off both the paraffin and the argols, dissolving the cream of tartar in boiling water, and allowing it to crystallize on cooling. The paraffin may also be recovered, as it merely melts and rises to the surface where it solidifies on cooling. The inside surface of the scraped tank is, when dry, again coated with paraffin.

It is common commercial practice in California to store distilling material (wine of poor quality to be used later for distilling) in new concrete vats in order to "cure" the surface.

Bioletti (1934) has recommended that new cement tanks be treated essentially in the manner described by Fabre, namely by washing with strong tartaric acid solution.

Glazed porcelain or glass tiles have been used as linings for concrete tanks, but ordinarily the cement used in attaching and joining the tiles is attacked; wine then penetrates behind them, where it sours when the tank is emptied and becomes a source of contamination of wine subsequently stored in the tank.

Many protective coatings, a number of them patented, have been devised for concrete surfaces, although most of these are unsuitable for wine tanks either because of toxic constituents, such as lead salts, hydrofluoric acid, or hydrofluosilicic acid, or because they contain substances, such as linseed oil, partially volatile odoriferous petroleum solvents, turpentine, etc., that adversely affect the flavor of wine.

EXPERIMENTAL PROCEDURE

The following experiments were conducted in order to compare the relative resistance of various cements to the solvent action of wine and to determine the suitability of various protective treatments and coatings.

Comparison of Various Cements

Eight cements were used in preparing standard briquettes one inch thick, such as are used for cross sectional strength tests. One part of cement, three parts of Ottawa sand, and the proportion of water recommended by the Civil Engineering Department of the University of California for each respective cement were mixed under standard conditions, pressed into molds, stored in a moist chamber for

about 24 hours, and cured under water for four weeks. The cements were of the following types:

C-1 Normal Portland cement
C-2 Low heat Portland cement
C-3 High early Portland cement
C-4 General purpose Portland cement
C-7 80 per cent of C-4 and 20 per cent puzzolan
C-8 70 per cent of C-4 and 30 per cent puzzolan
C-58 Santa Cruz normal Portland cement
C-62 Santa Cruz high silica cement.

The chemical composition of the cements was furnished by the Civil Engineering Department but will not be given in detail here. The CaO content of the "regular" cement ranged from 55.66 to 65.60 per cent; the Fe_2O_3 from 2.38 to 4.94 per cent; SiO_2 from 21.08 to 27.74 per cent; and Al_2O_3 from 4.64 to 7.26 per cent.

The puzzolan cement used in C-7 and C-8 contained 71.14 per cent CaO, 44.18 per cent SiO_2, 17.45 per cent Fe_2O_3, and 16.44 per cent Al_2O_3. It was very high in SiO_2.

The briquettes were placed in quart fruit jars of dry red wine (Claret type) and dry white wine (so-called Riesling type) and were stored at room temperature, 18.3 to 21.1°C. (65 to 70°F.), in the filled, sealed jars. The wines were analyzed after two and after ten days' storage for calcium and iron, and after about three months' storage. for iron only (Table 1). Calcium was determined on a 100-c.c. sample by evaporating to dryness in a Kjeldahl flask, wet ashing with H_2SO_4 and $HC10_4$, diluting and precipitating Ca as the oxalate, dissolving in H_2SO_4, and titrating was standard $KMnO_4$ solution. Iron was determined by the orthophenanthrolene, colorometric described recently by Saywell and Cunningham (1937).

Much insoluble calcium tartrate accumulated in the bottoms of the jars and it is probable that the supernatant clear wine was saturated to supersaturated with calcium tartrate and malate, probably accounting for the rather erratic changes in Ca content between the fifth and tenth days of storage of the red wine. After three months' storage the wines were neutral to alkaline in reaction, favoring the precipitation of iron, which probably accounts for the observed decrease in iron content on prolonged storage.

Most of the cements caused relatively slight increase in iron content of the two wines. On the other hand, the calcium increase was large, sufficient under the conditions of the tests to eventually completely neutralize the acidity of the wine; most of the Ca which had taken part in the reaction then appeared as a precipitate of very slightly soluble, organic Ca salts. In commercial practice the ratio of

surface of the concrete to the volume of wine is much lower than in these experiments; hence, there would be much less Ca dissolved. Thus, a tank 16x16x16 feet would hold approximately 30,500 gallons and the ratio of volume to area would be approximately 20 gallons per square foot; whereas in our laboratory tests the ratio was only 1.14 gallons per square foot. Nevertheless, as pointed out by Fabre (1920), there is danger of dissolving sufficient lime under practical conditions to impair the quality of the wine and possibly sufficient

TABLE 1

Calcium and Iron Content of Dry Wines After Storage in Contact With Briquettes Made With Various Cements

Sample	After 5 days' storage		After 10 days' storage		Total acid per 100 c.c. at 10 days	Fe after 3 months' storage
	Ca	Fe	Ca	Fe		
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*gm.*	
White untreated	69	4.0	.....	.....	.58	.....
C-1 white	124	7.5	276	7.5	.45	5.9
C-2 white	145	9.6	277	9.6	.47	6.8
C-3 white	132	5.8	249	5.5	.52	.....
C-4 white	119	7.3	260	7.6	.51	.....
C-58 white	237	7.8	431	8.8	.51	3.2
C-62 white	162	5.3	276	5.8	.32	2.5
C-7 white	162	7.8	299	28.1	.39	4.3
C-8 white	193	5.6	438	5.5	.37	2.4
Red untreated	75	3.4	.....	.....	.62	.....
C-1 red	229	5.0	320	12.4	.36	2.1
C-2 red	271	4.7	311	5.9	.33	3.5
C-3 red	227	4.2	196	8.5	.26	4.1
C-4 red	181	4.1	185	8.1	.32	5.2
C-58 red	1091	4.4	281	7.8	.30	.....
C-62 red	195	4.3	408	5.4	.32	.....
C-7 red	597	4.8	278	9.5	.32	4.3
C-8 red	1386	4.4	322	9.0	.....	.....

iron in white wines to cause haziness or cloudiness owing to precipitation of colloidal compounds of iron (iron casse).

In a second experiment test cylinders four inches long and two inches in diameter were prepared, as previously described, from 14 Portland cements. They were cured in a moist chamber for 28 days; stored in red dry wine for 36 days, as previously described; and the iron content of the wine in each container was determined. The cements used varied in Fe_2O_3 content from 1.91 to 6.66 per cent. The original wine contained 7.9 p.p.m. of iron. In only three cases was there an increase in iron content, the increase being 2.3, 3.2, and 3.2 p.p.m. In the other 11 cases the iron content decreased, the decrease ranging from .5 to 4.8 p.p.m. Probably these cylinders were stored in

the wine beyond the period of maximum iron pick-up with the result that, owing to marked decrease in acidity by the lime dissolved from the cement, some iron precipitated. Iron decrease on long storage is shown (Table 1). Upon removing these cylinders from the wine it was observed that they were heavily coated with tartrates, and also that they had become black in color from the absorption of wine pigments on the surface. The black color was probably caused by formation of an iron tannate. This observation indicates that treatment of the fresh surface with tannin solution might be desirable in order to reduce the tendency for iron to dissolve in the wine.

Comparison of Protective Coatings and Treatments of Concrete

In qualitative experiments made in 1915 in this laboratory it was found that paints and coatings containing lead compounds were attacked severely by wine, rendering it very metallic in taste and probably very toxic. In tests also made at that time and repeated recently, it was found that coatings containing linseed oil imparted a disagreeable flavor and odor to the wine. Other qualitative tests were made with sodium silicate coatings applied at various dilutions and in some cases followed by treatment with dilute tartaric acid solution after the silicate coatings had dried. Usually the coatings became crumbly or flaked off, and in all cases where the cylinders were later stored in wine there was severe reaction. Some test cylinders were also coated with dilute casein solutions (casein dissolved in dilute NH_4OH and excess NH_3 removed by boiling), with and without subsequent treatment with dilute tartaric acid or dilute formaldehyde. Adherence was poor, the coating becoming friable or flaking off. Coatings consisting of various mixtures of beeswax, rosin, and paraffin were firm and of good appearance when first applied, but after contact with wine for a few weeks, they blistered badly and flaked off. They were wholly unsatisfactory.

In the first quantitative experiment made in 1936 the following protective coatings were applied to well-cured, dry, four-by-two-inch test cylinders made of one part normal Portland cement to three parts of sand. The coatings were (1) a commercial beer pitch, (2) special beer pitch, (3) a commercial black roofing paint (asphalt base), and (4) Bass-Hueter Company odorless black enamel for wine tanks. At the end of one week's storage there was no increase in iron content of white wine stored in contact with Coatings 1, 2, and 3, the iron content being within the experimental error (about .50 p.p.m.). The red wine increased slightly in iron content, namely from an original of 3.4 p.p.m. to 5.3, 4.7, and 5.3 p.p.m. in contact with Coatings 1, 2,

and 3 respectively. No analyses were made of wine in contact with Coating 4 at that time. After one week's contact with the wine, Coatings 1, 2, and 3 were badly blistered and the calcium content of the wines had increased several fold. Coating 4 was in good condition.

After about three months' storage, Coatings 1, 2, and 3 were in very badly blistered condition while No. 4 was in fairly good condition, showing only a somewhat roughened surface but no blistering or scaling. The iron contents of the wines at this time were as follows: (1) in red wine 7.8 p.p.m., (2) in red wine 7.3 p.p.m., and (4) in red wine 4.0 p.p.m. The original wine contained 3.4 p.p.m. of iron. Coating 4, the Bass-Hueter Company black enamel, has been used quite extensively as a coating for cement wine tanks and steel wine-tank cars commercially.

In a second experiment 21 different coatings were applied to test cylinders, which were then stored in red dry wine. Observations on the condition of the coatings and taste and odor of the wines were made at the end of four to six weeks. Calcium determinations were also made (Table 2).

The coatings were applied in three ways, namely to untreated test cylinders, to others previously treated with one washing with 25-per cent tartaric acid, and to others treated first with .5-per cent tartaric acid and then with 25-per cent tartaric acid. The acid-washed cylinders were allowed to stand several days and were dry when painted.

After painting or otherwise coating, the cylinders were allowed to stand for a week or longer at room temperature to permit evaporation of solvents or thinners and hardening of the coatings..

The coatings composed of Gilsonite plus paraffin gave the most satisfactory results. While that composed of equal proportions of Gilsonite and paraffin was firmer and somewhat more impervious than that consisting of 75 to 25 per cent of paraffin, the latter was much less difficult to apply since its melting point is sufficiently low to permit normal use of a paint brush. Recently, a large wine-producing company coated a number of its concrete, wine-storage tanks with the 75-per cent paraffin and 25-per cent Gilsonite mixture, and it reports very satisfactory results to date. However, we found that wine stored for about six months in jars in contact with concrete cylinders so coated acquired a noticeable "paraffin" taste. In this case, however, the ratio of surface of coating to volume of wine was very much greater than in a large concrete tank. Judged by the amount of calcium increase in the wine, the Gilsonite-paraffin mixtures were superior to the other coatings. Used under practical conditions the only criticism made of them has been their tendency to become brittle and

to crack if treated roughly or if wine refrigerated to near the freezing point comes in contact rapidly with the coating. Gilsonite is a brittle, naturally occurring, black asphaltum. It is practically tasteless and odorless. It may be heated to a high temperature to expel the small amount of remaining volatile, odoriferous compounds before use if desired.

The paraffin coating resisted action of the wine fairly well; although for some reason it appeared to be rather permeable to the wine, as the Ca content of the wine in contact with paraffined cylinders increased several fold in four to six weeks.

Of the commercial preparations tested in this experiment, Bass-Hueter black enamel proved best. After about six weeks' contact with the wine it had become rough and slightly crumbly. The flavor and odor of the wine were not noticeably affected. The rather large increase in Ca content of the wine, however, indicates that the coating was not entirely impervious to penetration of the wine to the concrete surface.

None of the other coatings were satisfactory; they became either blistered or crumbly or imparted disagreeable tastes and odors to the wine. The coatings containing an asphalt base, however, in most cases remained in good condition.

It is evident that treatment of the concrete with tartaric acid before coating markedly decreases the amount of calcium dissolved by the wine from the concrete. Its use is strongly recommended, whether or not the concrete surface is given any other treatment or coating.

Qualitative tests were also made with the following materials applied in two coats:

1. Asphalt emulsion, Pioneer Flintkote Company, San Francisco.
2. Tournesit dissolved in a colorless, volatile, petroleum product solvent.
3. Two experimental paints, Paraffin Paint Company.
4. Protectokote, Sterling Paint Company.
5. Two Bakelite preparations.
6. S-O floor hardener, Standard Oil Company of California.
7. Bass-Hueter black enamel, National Lead Company and Bass-Hueter Company.

The asphalt emulsion proved very satisfactory since it adhered well and did not impart any noticeable odor or taste to the wine. It is an emulsion of water, an inorganic colloid, and finely divided, tasteless asphaltum. As furnished for these tests it was about the consistency of axle grease or cup grease. It was applied by brush or by spatula.

TABLE 2

Comparison of Several Protective Coatings With Respect to Effect on Flavor and Calcium "Pick up"

Coating	Condition of wine and coating after storage	Ca increase with untreated cylinders	Ca with cylinders treated with 25% tartaric acid	Ca with cylinders treated with .5% and 25% tartaric acid
		p.p.m.	*p.p.m.*	*p.p.m.*
1. 50% Gilsonite and 50% paraffin.	Coating in excellent condition. Slight paraffin taste.	2.8	2.1	1.7
2. Paraffin applied hot.	Some calcium tartrate crystals. Slight paraffin taste.	22.8	38.8	17.4
3. Bass-Hueter black enamel.	Some roughening of coating. Flavor satisfactory.	65.3	21.1	18.4
4. W. P. Fuller Co. No. 7296. Clear, thin cellulose acetate lacquer.	Strong taste of solvent. Some pitting; no blistering.	72.7	34.7	23.3
5. W. P. Fuller No. 7464. Clear lacquer.	Strong taste of solvent. Coating badly attacked.	21.9	55.7	31.9
6. W. P. Fuller No. 7462. Clear lacquer.	Strong taste of solvent. Coating badly attacked.	37.8	24.8	8.3
7. W. P. Fuller No. 7447.	Strong off-odor and taste. Coating badly blistered.	27.9	No data	11.2
8. W. P. Fuller No. 7463.	Strong off-odor and taste. Some action of wine on the coating.	41.4	28.7	45.6
9. W. P. Fuller No. 7065.	Considerable roughening of coating. Flavor fair.	40.9	17.2	21.7

TABLE 2 *(Concluded)*

10. W. P. Fuller No. 7021. A pitch-type coating applied in molten condition.	Some off-odor and taste. Coating badly blistered.	56.5	No data	38.7
11. Kotite. An asphalt base paint.	Strong off-odor and taste. Considerable flaking.	98.8	33.2	No data
12. 25% Gilsonite and 75% paraffin.	After 6 months, a slight paraffin taste. Coating in excellent condition.	7.4	12.1	1.1
13. Super Beckacite No. 2000. Synthetic resin in solution.	Strong solvent odor and taste. Considerable action on coating.	68.7	24.9	31.1
14. Super Beckacite No. 1001. Synthetic resin in solution.	Strong solvent odor and taste. Considerable action on coating.	Above 90	17.5	40.7
15. Super Beckacite No. 1003. Synthetic resin in solution.	Strong solvent odor and taste. Considerable action on coating.	42.7	58.9	25.6
16. Synthetic copal ester gum in solution.	Some off-taste. Considerable damage to coating.	47.0	11.2	17.7
17. Pyroflex dissolved in light petroleum solvent.	Considerable color dissolved by the wine. Coating badly attacked.	20.1	58.9	29.3
18. Beer pitch. Commercial No. 1.	Badly blistered. Flaked off.	1540	Excessive	Excessive
19. Beer pitch. Commercial No. 2.	Badly blistered. Flaked off.	3282	Excessive	Excessive
20. Black paint. Rubber base. Commercial.	Badly blistered. Taste of solvent.	3780	Excessive	Excessive
21. Parex black paint.	Badly blistered. Taste of solvent.	21.9	No data	No data

The water rapidly evaporated, leaving a continuous, tough, asphaltum coating. The second coat was applied after the first coat had dried thoroughly. The coating remains slightly "tacky" and is not as hard as the Gilsonite-paraffin coating but is tougher.

The other products adhered well and were quite resistant to the destructive action of the wine, but all except the Bass-Hueter black enamel imparted disagreeable odors and tastes.

At the suggestion of the producer a small amount of cement in one test was added to the asphalt emulsion in order to make the coating firmer. While it accomplished this object, much Ca was dissolved from the cement by the wine. Therefore, if it is used in practice it would be advisable to treat the coating thoroughly with tartaric acid solution

TABLE 3

Increase in Iron Content of Wine Stored in Cement With Black Iron Test Pieces Coated in Various Ways

Sample	Iron content of wine	Iron increase in wine
	p.p.m.	*p.p.m.*
1. Control in glass	6.4	0
2. Pyroflex	43.0	36.6
3. 50% paraffin + 50% Gilsonite	14.4	8.0
4. Kotite	16.6	10.2
5. Goodrich No. 1009	10.0	3.6
6. Goodrich No. 1031	10.4	4.0
7. W. P. Fuller No. 7464	36.5	30.1
8. W. P. Fuller No. 7021	21.0	14.6
9. Bass-Hueter black enamel	8.0	1.6

before storing wine in a tank. One large company has coated the interior of all its concrete and wooden tanks with asphalt emulsion and has found it very satisfactory. It can be applied to a wet surface equally as well as to a dry surface.

It is possible that if a completely volatile or tasteless solvent could be found for some of the synthetic preparations, such as Tournesit, one or more satisfactory coatings could be found. Acetone or alcohol-soluble lacquers, such as the Bass-Hueter enamel, do not present this problem. Apparently, however, judging from present results, these coatings are not completely resistant to action of the wine and they gradually deteriorate.

Tests of Coatings for Steel Containers

Thoroughly cleaned sheets of black iron four by two inches in size were given three coats of the materials listed (Table 3). The coated sheets were stored in red dry wine for 43 days and the wines were then analyzed for iron content.

Pyroflex, as on concrete, was seriously attacked by the wine. The paraffin-Gilsonite coating was in good condition. Kotite imparted a disagreeable odor and taste and was seriously attacked by the wine. Goodrich 1009 softened but Goodrich 1031 remained in good condition; both imparted a disagreeable odor and taste to the wine. W. P. Fuller 7464 and 7021 gave a disagreeable odor and taste to the wine and were seriously attacked. The Bass-Hueter black enamel was in good condition, except for some roughening. It gave the best results of the coatings tested with respect to iron increase in the wine. It would probably have given even better results if the test pieces coated with it had been given a coating of paraffin or asphalt emulsion or 25 per cent Gilsonite plus 75 per cent paraffin. A qualitative test with asphalt emulsion gave promising results and is worthy of further trial. One large shipper of wine paints the inside of steel shipping tanks with Bass-Hueter black enamel and covers this surface with paraffin; he reports good results.

SUMMARY AND CONCLUSIONS

1. Wines dissolved but little iron from concretes made of 22 different cements, some of which were very high in Fe_2O_3. In white dry wine, however, enough iron was dissolved in some cases probably to render the wine unstable with respect to clouding. The calcium "pick-up" was high with all free cement surfaces. The problem appears to be one of protection of the concrete against dissolving of calcium rather than of iron.

2. Treatment of the concrete surface with strong tartaric acid solution greatly reduced the amount of calcium dissolved by the wine. Two treatments, one with .5-per cent and one with 25-per cent solution gave better protection than one treatment with 25-per cent solution. A coating of insoluble calcium tartrate is formed.

3. Of the commercial preparations tested in these experiments Pioneer Flintkote asphalt emulsion and Bass-Hueter black enamel gave the best results.

4. Beer pitches and experimental mixtures of melted beeswax, paraffin, and rosin blistered badly and gave very little protection.

5. Water-glass coatings failed to adhere satisfactorily, or did not prevent excessive reaction between the wine and concrete.

6. Gilsonite, 25 per cent, plus paraffin, 75 per cent, gave a highly protective coating. It is inexpensive and easily applied but is somewhat more brittle than the asphalt-emulsion coating.

7. The tests of coatings for steel were less extensive than with concrete. Of the coatings tested, a Gilsonite-paraffin mixture and Bass-

Hueter black enamel gave the best results. Asphalt emulsion appears very promising, from qualitative tests not reported on in detail in this paper.

8. There is opportunity for much improvement in commercial preparations and need for further trial under large-scale commercial conditions of the most promising of the various coatings reported on here.

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A THERMOSTATICALLY CONTROLLED CABINET FOR USE IN ETHYLENE TREATMENT OF PEARS

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(Received for publication, July 23, 1937)

During a recent study of the utility of ethylene in the ripening of pears, it became necessary to construct air-tight cabinets of special design. Inasmuch as these cabinets proved very successful for the purpose, the details and specifications may be of interest to those engaged in similar researches. A front view of the outside of the cabinet shows the hinged door opening downward, ventilating flue, and understructure (Fig. 1). A view of the interior of the cabinet shows the method of confining the pears in studies involving different gaseous environments, using five-gallon wide-mouthed bottles provided with tubes through the air-tight covers to permit charging the bottles with gas and withdrawal of gas samples for study (Fig. 2).

The cabinets were constructed of one-inch Celotex (equivalent to .88 of an inch of cork in insulation value) and lined with 26-gauge, galvanized sheet metal, leaving an air space of seven-eighths of an inch between the Celotex and the metal, thus insuring sufficient insulation for the temperatures employed in the study of ethylene treatment of pears, i.e., between 15.6 and 32.2°C. (60 and 90°F.). The method of wiring with the use of one-half-inch conduit pipe and the details of construction are shown (Figs. 3 and 4). The inside dimensions of the cabinets were 45x27x29 inches, thus providing sufficient space to hold several "lugs" of fruit.

A false bottom of "transite" one-fourth of an inch thick, perforated with three-fourths-inch holes and mounted four inches above the true bottom, permitted free circulation throughout the cabinet. Openings one and one-eighth inch in diameter were placed in the ends, back, and bottom of the cabinet to permit withdrawal of gas samples, insertion of potentiometer electrodes, etc. (Fig. 3). These openings were constructed with the use of one-and-three-eighths-inch brass pipe soldered to the inner jacket and when not in use were plugged with corks. The door of the cabinet was similarly constructed with the use of Celotex and galvanized sheet metal. A long double glass window was built into the door to permit observation of the interior of the cabinet. The edges of the door were of wedge-shape construction covered with felt to assure an air-tight seal when drawn up tight

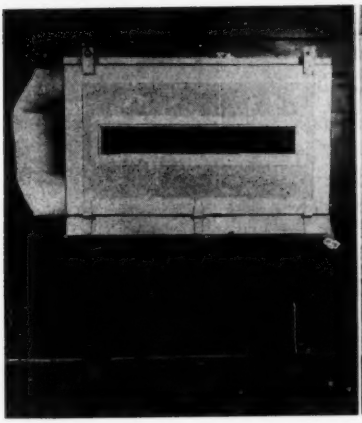

FIG. 1.

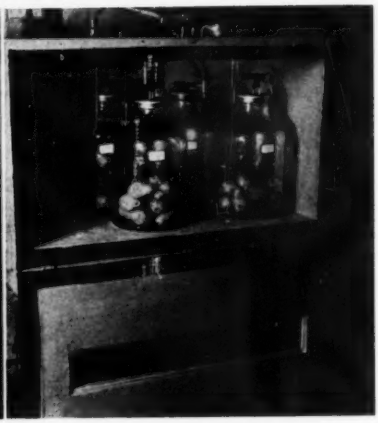

FIG. 2

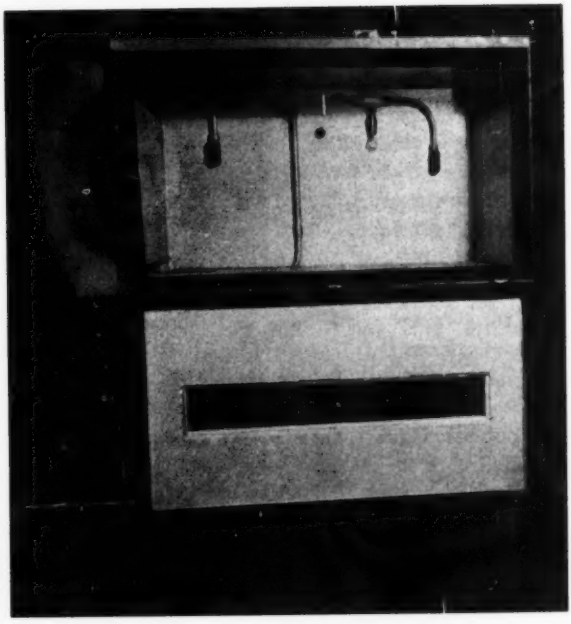

FIG. 3.

with wing nuts at the top. Hinged at the bottom, the door permitted free and easy access to the inside of the cabinet; details of the corner and door construction are given (Fig. 5).

An eight-inch circulating flue of galvanized sheet metal was installed at the end of the cabinet. This was thoroughly insulated with sheet asbestos built up to seven-eighths of an inch in thickness and covered with cheesecloth (Fig. 6). A six-inch fan was built into the

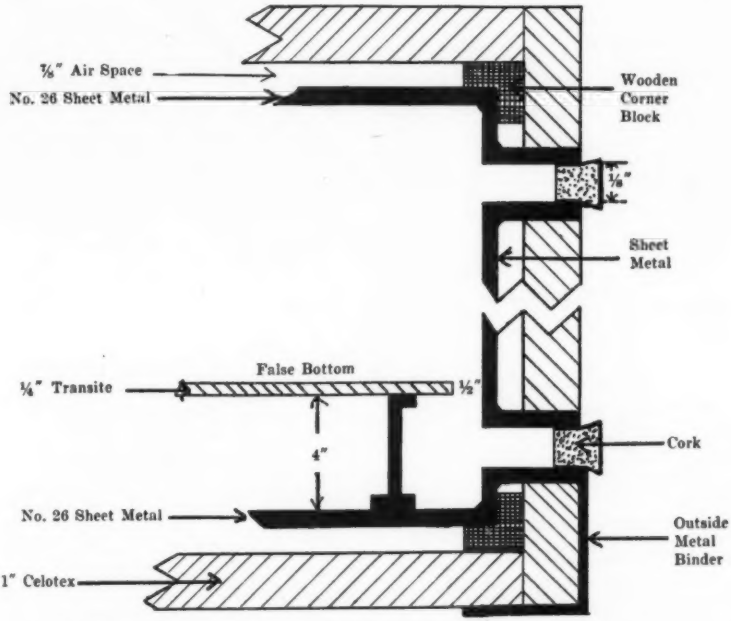

FIG. 4. Cross section of cabinet, showing details of construction.

upper portion of the flue to circulate the air, thus obviating the possibility of "dead" air pockets in the cabinets. The fan was fashioned from an ordinary six-inch electric fan by removal of the guard and base. It was suspended in the circulating flue by a special rubber mounted fixture (Fig. 6). The speed of the fan was reduced by means of a "fixed" rheostat made from "Nichrome" wire, adjusting the circulation of air within the cabinet to 360 cubic feet per minute.

The cabinet was heated with two 220-watt Westinghouse non-glow space heaters installed at the base of the circulating flue directly underneath the fan. The temperature within the cabinet was controlled to plus and minus 2°F. with the aid of a DeKhotinsky Bi-

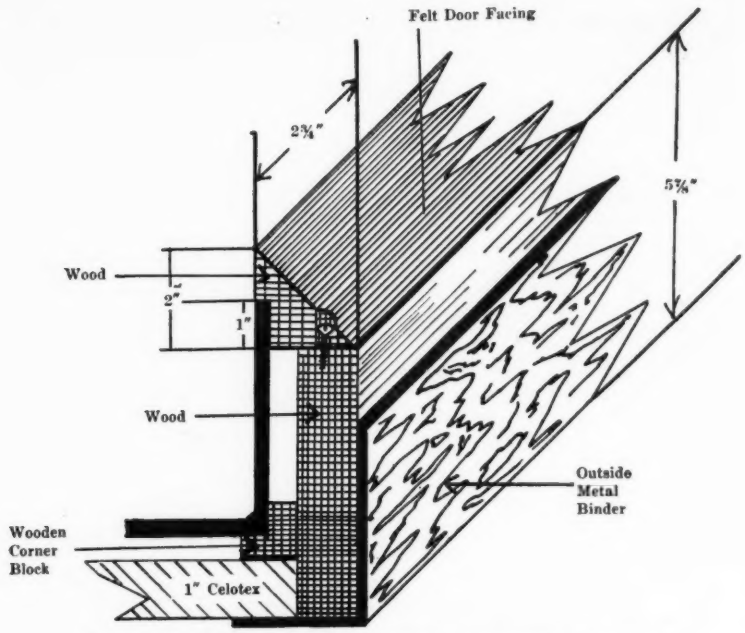

FIG. 5. Lower left front corner, showing construction of corner and door facing.

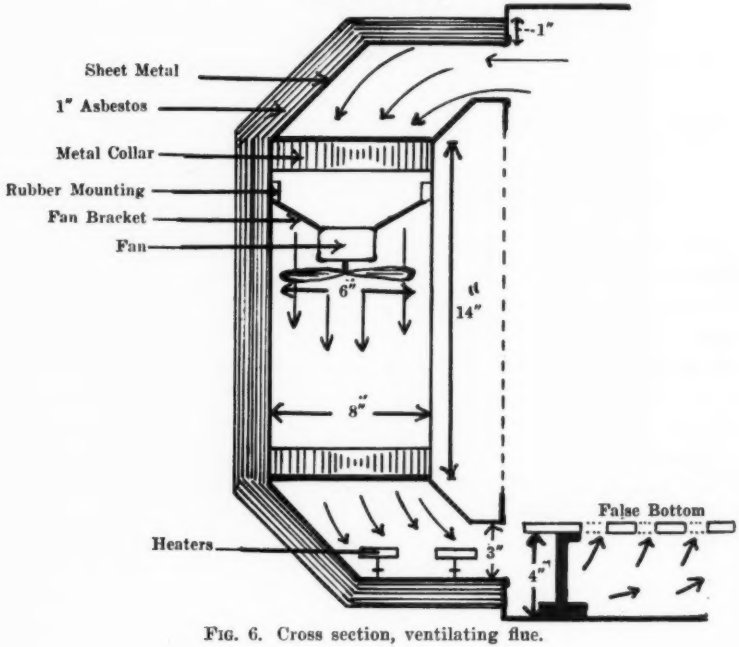

FIG. 6. Cross section, ventilating flue.

metallic Thermoregulator installed in the top. Two condensers of 25 mfd capacities were placed in the thermoregulator circuit to prevent "arcing" of contacts (Fig. 7).

All the electrical wiring in the cabinet was enclosed in one-half-inch conduit pipes made air-tight by plugging with asphalt after insertion of the wires. Two double electrical outlets were installed to permit the use of electrical instruments within the cabinets. A 25-watt

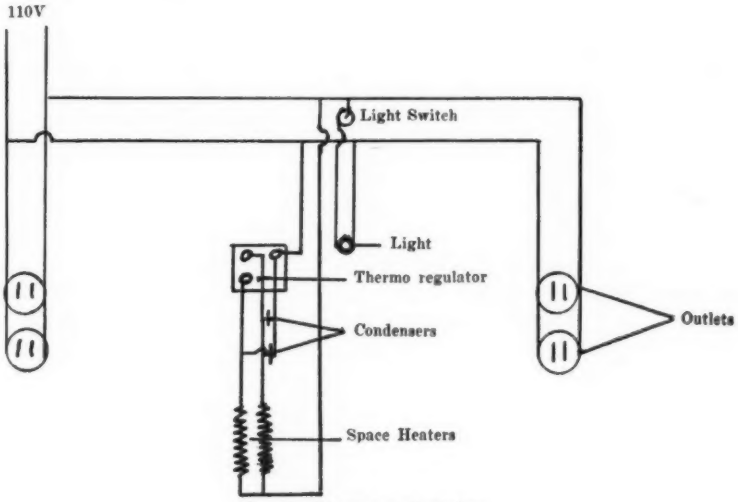

FIG. 7. Diagram of wiring.

light controlled by an outside switch was also included to permit the reading of instruments from the outside, thus obviating the necessity of opening the door.

The cabinets were mounted on a frame constructed from three-fourths-inch black iron pipe (Fig. 1). The leg supports were mounted on two-and-one-half-inch rubber-tired castors which made the moving of the cabinets easy.

This type of cabinet proved to be very satisfactory for the fruit-ripening work and also for the series of low-temperature studies subsequently conducted.

IDENTITY OF SUGARS IN THE RIPE BANANA

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(Received for publication, July 24, 1937)

Although the yellow banana of commerce *(Musa sapientum)* is a typical carbohydrate fruit, there seems to be no definite qualitative evidence in the literature concerning identity of the banana sugars. Approximately 80 per cent of the total solids in the green banana is starch, which is almost completely converted to various mono- and di-saccharides during ripening. These sugars have usually been considered merely as "reducing sugars" and "nonreducing sugars," and most investigators have reported them as such. Wehmer (1911) reported quantitative data, however, for the sucrose, glucose, and fructose content of ripe bananas but made no reference to the methods used. More recently Widdowson and McCance (1935) have considered the reducing sugars of the banana as glucose and fructose since the sum of these two sugars, determined separately, was sufficient to account for all of the reducing activity of the banana extract before inversion. Bailey (1912) came to the conclusion that maltose was not present among the sugars of the banana.

The purpose of this study was to subject the sugars of the ripe banana to a direct qualitative examination and to identify the various sugars found to be present.

EXPERIMENTAL METHOD

The fruit selected for analysis was of the Gros Michel variety, the ordinary yellow banana of commerce in the United States, which had been normally ripened. Two kilograms of pulp were macerated, centrifuged, and decanted. The viscous liquid obtained was immediately treated with neutral lead acetate to remove tannins, pectins, and proteins. Excess lead was removed by the addition of potassium oxalate and subsequent filtration. The filtrate was clear and sparkling, but after concentration *in vacuo* (49 mm.) at 70°C.(158°F.) it resembled molasses. This syrup reduced Fehling solution, exhibited an increased reducing power when inverted with citric acid, and showed a still greater reducing power when inverted with hydrochloric acid. Aliquots of this syrup were subjected to qualitative analysis for the identification of common, naturally occuring sugars.

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SUCROSE

There are no characteristic qualitative tests for detecting sucrose in the presence of other sugars. Therefore, the only certain means of identification was to precipitate the sucrose as a saccharate and to determine the optical and chemical properties of the sugar after liberation from this salt.

After several preliminary attempts, the Deguide process, used by Vrancken (1924), was employed to obtain pure sucrose. An aliquot of the original syrup was diluted with water, and barium hydroxide equivalent to four times the weight of the reducing sugar present was added. The mixture was boiled to decompose the reducing sugars and continued until the specific gravity of the solution reached 20 Baumé. More barium hydroxide, in the estimated proportion of one and one-half parts to one part of sucrose, was then added. This solution was held for 10 minutes at 80 to 82°C.(176 to 179.6°F.), after which the precipitated barium saccharate was decomposed by bubbling carbon dioxide through the suspension. After removing the barium carbonate by filtration, the solution was twice treated with barium hydroxide as before. The final filtrate was concentrated to saturation and treated with a relatively large volume of alcohol. The resulting crystalline precipitate was purified by several re-crystallizations from water and finally dried over sulphuric acid. Microscopic examination showed the crystals to be characteristic of sucrose. The specific rotation was found to be +66.3° at 20°C.(68°F.) (pure sucrose, = +66.5°). Solutions of the crystals did not reduce Fehling solution until after acid inversion. Using the modified Lane and Eynon method, as given in A.O.A.C. Methods of Analysis (1936), for invert sugar, it was found that after inversion the crystals had a reducing potency toward Fehling solution almost equal to that of a standard sucrose solution. One gram of the crystals had a reducing value equal to .9434 gram of pure sucrose. There seemed little doubt that the isolated crystals were actually sucrose.

MALTOSE

The observed differential inversion between hydrochloric acid and citric acid, previously mentioned, was a preliminary indication of the presence of maltose as shown by Haas and Hill (1921). To substantiate this, an aliquot of the original syrup was heated with an excess of phenylhydrazine acetate. The solution was filtered hot and the filtrate, after cooling, was found to contain a small quantity of yellow crystals. These were filtered, washed with cold water, taken up in boiling water, immediately filtered again, and allowed to crystallize. This purification was repeated until the melting point of the osa-

zone became constant. This melting point was found to be 204°C. (399.2°F.), which compared very well with the melting point (206°C.) of the osazone prepared from pure maltose. A mixed melting point determination also indicated that the sugar was maltose.

Because of the very low yield of maltosazone and because Bailey's (1912) examination of banana sugars had failed to reveal the presence of maltose, a second extract of two kilograms of banana was made solely for the purpose of identifying maltose. Approximately .4 gram of osazone was finally isolated. This osazone melted with decomposition at 205°C.(401°F.), showed a crystal structure characteristic of maltosazone, and contained 10.70 per cent nitrogen (10.76 per cent theoretical for a disaccharide osazone) as determined by a semi-micro Kjeldahl determination.

Bailey's failure to find maltose in bananas is not surprising. In our experiments the low yield of maltosazone indicated that maltose was present in very small quantities. Since a 10-per cent yield of maltosazone from maltose may be expected as indicated by Browne (1912), the separation of .4 gram of osazone from two kilograms of pulp indicated an original maltose content of .2 per cent, assuming that none of this sugar was lost by hydrolysis during extraction or mechanically during isolation and purification of the osazone. It is probable that maltose, an intermediate product in the enzymatic hydrolysis of starch to monosaccharides, is quickly hydrolyzed after its formation, according to Browne. Consequently the quantity of this sugar present at any time is probably fairly constant and small.

FRUCTOSE

Tests upon aqueous solutions of the original syrup with Seliwanoff's and Pinoff's reagents gave partial proof of the presence of fructose. The methylphenylosazone reaction, however, was believed to offer the best means of proving the presence of this sugar. Ten milliliters of the original syrup were treated with five grams of methylphenylhydrazine and a sufficient quantity of 95 per cent alcohol to give a clear solution. After warming it slightly and allowing it to stand 24 hours, the mixture was filtered. Four milliliters of 50 per cent acetic acid were added to the filtrate, the solution was heated on a water bath for 10 minutes, and then allowed to stand for 24 hours. The reddish-yellow crystals thus obtained were filtered and purified by several crystallizations from chloroform. The purified crystals had a melting point of 157.5°C.(315.5°F.) as compared with 160°C.(320°F.) for the methylphenylosazone obtained from pure fructose. The structure of both sets of crystals was identical when examined under the microscope.

GLUCOSE

The identification of glucose was carried out by the synthesis of glucose-pentabenzoate and glucose-diphenylhydrazone. A solution of the original syrup was shaken with benzoyl chloride in the presence of 20 per cent sodium hydroxide. Six parts of benzoyl chloride and 48 parts of alkali were added for each part of glucose assumed to be present. The mixture was placed in an ice bath for 24 hours. The precipitate which formed was filtered, shaken with 95 per cent alcohol, and again filtered. After evaporation at room temperature, colorless, needle-like crystals were obtained. These crystals, after purification by re-crystallization from alcohol, melted at 180°C.(356°F.). Glucose-pentabenzoate obtained from pure glucose had a melting point of 179°C.(354.2°F.).

The diphenylhydrazone was prepared by adding one and one-half parts of an alcoholic solution of diphenylhydrazine to a quantity of the original syrup estimated to contain one part of glucose. After standing for several days, small, colorless, prismatic crystals were obtained. Repeated re-crystallizations from hot water yielded the pure diphenylhydrazone with a melting point of 162.5°C.(323.9°F.). The diphenylhydrazone obtained in a similar manner from pure glucose melted at 161.7°C.(322.3°F.).

OTHER SUGARS

Aliquots of the original syrup were tested for the presence of all the other natural sugars for which there exists a characteristic qualitative reaction.

As a result of distilling ripe banana pulp with hydrochloric acid, .03 per cent of furfural was formed. This indicated the presence of either pentoses or pentosans to the amount of .05 to .06 per cent. The banana pulp extract, however, showed no appreciable furfural upon distillation with hydrochloric acid and gave negative tests with orcin and naphthoresorcin. In all probability pentose sugars are not present in banana pulp except in combination as pentosans.

The other sugars tested for and the tests used are given below. In all cases the results were negative, indicating the absence of these sugars.

d—mannose	phenylhydrazone
d—, l—, and dl galactose	mucic acid reaction
d—, sorbose	osazone
d—, and l— mannoheptose	phenylhydrazone and osazone
raffinose	attempted isolation and mucic acid reaction
melezitose	acetylation
rhamninose	mucic acid reaction

SUMMARY

An identification of the sugars present in the pulp of ripe bananas was made by preparation of characteristic derivatives or by actual isolation of the individual sugar.

The principal sugars of the ripe banana were definitely shown to be sucrose, maltose, fructose, and glucose. Maltose is present in very small amounts.

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INSTANT WHIPPING OF CREAM BY AERATION

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(Received for publication, July 26, 1937)

INTRODUCTION

Cream whipping, as ordinarily practiced, is the process of agitating cream, usually of not less than 30 per cent butterfat, in such a manner that the volume is increased through the entrapping of gases (usually air) in the form of small bubbles. Sufficient agitation is necessary to bring about partial clumping of the fat globules in order to retain the incorporated gas. Various types of mechanical devices have been developed for the purpose of facilitating the whipping process. Even with the best devices, however, cream will not whip satisfactorily when conditions are unfavorable; for example, when the temperature is too high, fat clumping becomes so pronounced that buttering occurs. Babcock (1922) lists seven factors that may affect the whipping ability of cream: pasteurization, homogenization, composition, temperature, acidity, sugar content, and foam stabilization.

The process of whipping cream by aeration departs markedly from these mechanical methods. By way of introduction to this process it may be stated that the fat globules are not clumped during whipping, as the gas cells are generated within the body of the cream and are not incorporated by agitation. The gas cells are created by dissolving a soluble gas under pressure in the cream and then allowing the cream to flow out from under this pressure. As the cream flows through the valve of the pressure bottle, the gas cells are formed owing to a decrease in solubility of the gas with the decrease in pressure. Thus the whipping takes place by physical-chemical rather than by mechanical means. The process is discussed in greater detail in the following section.

DISCUSSION

General Method: Before presenting the experimental studies it seems that a discussion of the process and its practical application is necessary. As was stated in the introduction, the process depends on a physical-chemical method of forming the gas cells in the body of the cream. According to Henry's Law, the solubility of a gas in a liquid varies directly with the partial pressure of the gas. Therefore,

when cream is placed in a pressure bottle and gas is passed into it to some definite pressure, a certain amount of the gas will dissolve in the serum of the cream. If this pressure is doubled, approximately twice as much gas will dissolve in the cream; if the pressure is trebled, three times as much gas will dissolve. This shows that varying amounts of gas can be made to dissolve in a given quantity of cream by merely varying the gas pressure. If the cream is saturated at some elevated pressure with a soluble gas and then allowed to flow out to atmospheric pressure, the gas that was forced to dissolve owing to the elevated pressure comes out of solution in the form of small gas bubbles or cells uniformly distributed throughout the body of the cream. The increase in the volume (overrun) depends upon how much gas is thrown out of solution, which in turn depends upon what pressure was used in saturating the cream. The rigidity or stiffness of the whip depends upon many factors, the most important of which are percentage of butterfat, overrun, temperature of the cream, and age of the cream.

It should be pointed out that after the cream is saturated with the gas at this elevated pressure the cream is still in the liquid state. Also, all of the cream need not be dispensed from the bottle at one time, and only that which is dispensed is whipped. Thus, it is clear that this process enables one to produce very small quantities of whipped cream at a time, since less than a teaspoonful of whip may be allowed to flow from the bottle. The cream remaining in the bottle is in the liquid state ready to be dispensed instantly into whipped cream all at one time or in the amount and when needed.

Features of the Process: As has been pointed out, this process furnishes a means of producing whipped cream in small amounts as needed. This eliminates the waste that results when whipped cream is stored for intermittent use by wholesale users. Furthermore, the product is uniform in appearance and taste because it is whipped at the moment of serving. Owing to the fact that the whipping process is physical-chemical rather than mechanical in nature, whipped cream is formed even when whipping conditions are unfavorable.

Since cream instantly whipped by aeration is dispensed from a sealed syphon bottle directly on the dish being served and since the bottles are processed in dairy plants under sanitary conditions, this process furnishes the consuming public with a more sanitary whipped cream than they may obtain otherwise. Often the whipping equipment used in kitchens of restaurants and other public eating houses is not properly washed and seldom sterilized; furthermore, the whipped cream is usually stored in open dishes, from which it is dipped intermittently and thus subjected to still more contamination.

Practical Application: The process was used[1] in practice at the University of Illinois Dairy Manufactures Division as a commercial commodity and has been definitely established as a practical method.

In practical application small, steel, syphon bottles are charged with cream and gas under pressure in the processing plant. The charged bottles are delivered to the soda fountain, restaurant, or home where the whipped cream may be produced in the quantity desired by momentarily opening the valve of the bottle. The cream that flows out from under the gas pressure whips at the point of release of pressure. That which remains in the bottle stays in the natural liquid state until it too is allowed to flow out of the bottle. When the bottle is empty, it is returned to the processing plant where it is opened, washed, sterilized, and recharged for another trip to the consumer.

Discussion of Gas Used: The gas used to whip the cream is not air because of its low solubility; it should be soluble to between 100 and 150 volumes of gas at standard conditions in 100 volumes of cold water. The gas used should be odorless and tasteless as well as nontoxic. Since nitrous oxide (N_2O) meets these requirements, it is the gas used. The fact that nitrous oxide is used as an anesthetic in medicine requires that a short discussion of its physiological properties be presented.

Nitrous oxide, when used as an anesthetic, is mixed with oxygen, using between 90 to 95 per cent nitrous oxide and 5 to 10 per cent oxygen. The patient is soon rendered unconscious, but upon being allowed to breathe air he returns to consciousness almost immediately and in a few minutes all of his body functions are normal. Even when held under the anesthetic mixture for 15 to 20 minutes and then allowed to breathe air again the patient becomes conscious almost immediately and his body functions are normal in a few minutes. Green and Currey (1925) state that repeated administrations of nitrous oxide anesthesia over a period of months have no apparent ill effects. Desmarest and Lascombes (1921) state that a mixture of N_2O and O_2 never produces cholemia, indicating that this anesthetic does not affect the liver. Wieland (1922) states that phenomena of life which are not dependent upon the presence of oxygen are no more affected by N_2O gas than by any other indifferent gas. Teschendorf (1922) states that N_2O has no specific effect on rabbit intestines. Orcutt and Waters (1933) show that N_2O escapes from the body twenty times as fast as ethylene under conditions of anesthesia. As one

[1] It is now on sale in 30 of the larger cities in the United States by Aeration Products companies. Approximately a million bottles processed as described in this paper are used annually at the present rate of sale.

studies the literature, it becomes apparent that N_2O is highly recommended as an anesthetic because of its non-toxic effect on the system.

The amount of N_2O used to get a quart of whipped cream, even if used in an anesthetic bag, would not be sufficient to act as an anesthetic. The amount of gas eaten in the form of whipped cream is, by comparison, so small that there is no possibility of physiological effects since N_2O has no physiological effects except when there is a deficiency of oxygen.

Pressure Syphon Bottle: A special syphon bottle [2] was designed for the practical application of the process. A cross-sectional drawing shows the latest design of the bottle (Fig. 1); it has a capacity of one pint and is made of steel.

The base or bottle proper (1) is rolled in slightly at the top to give a broader gasket surface (2). The top is threaded on the outer edge (3) to allow the engagement of the large ring nut (4) which presses the top of the bottle (5) tightly against the rolled edge of the base, making a perfect seal by means of a rubber gasket (6). A specially designed Schrader valve unit (7) is fitted into the top (5) near the edge. To this Schrader valve unit is attached the small-bore tube (10) which extends very close to the bottom outside edge of the bottle. A dust cap (8) is used to protect and cover the upper, open end of the Schrader valve unit. The top of the bottle (5) has a semicircular depression stamped into it in such a manner that the edge of the ring nut completes the circle into which an ordinary milk-bottle cap can be pressed for labeling purposes. A dispenser (9) screws on to the projecting stem of the Schrader unit after the removal of the dust cap. When the button on top of the dispenser is pressed downward, it opens the Schrader valve, allowing cream to flow out of the container when it has been processed with cream and gas under pressure.

PLAN AND PROCEDURE OF EXPERIMENTAL WORK

The variables studied were (1) pressure of nitrous oxide, (2) fat content of the cream, (3) age of cream, (4) homogenization of cream, (5) increased viscosity of cream, and (6) addition of foam stabilizers to the cream.

The general procedure was to use the pint steel syphon bottle and, with the bottle open, to add one-half pint of cream-mix. The cream-mix was made up by adding six per cent of sugar to the cream and flavoring it with vanilla. The bottle, containing one-half pint of the mix, was closed tightly by using a bottle vise and wrench on the ring nut.

[2] The bottle was designed and constructed by A. H. Smith of the G. Frederick Smith Chemical Co., Columbus, Ohio.

The bottle was then inverted to give access, by means of the Schrader valve unit and attached tube, to the half-pint gas space above the half-pint of mix. It was charged to 90 pounds per square inch with nitrogen gas which is not soluble in cream. The valve was then opened

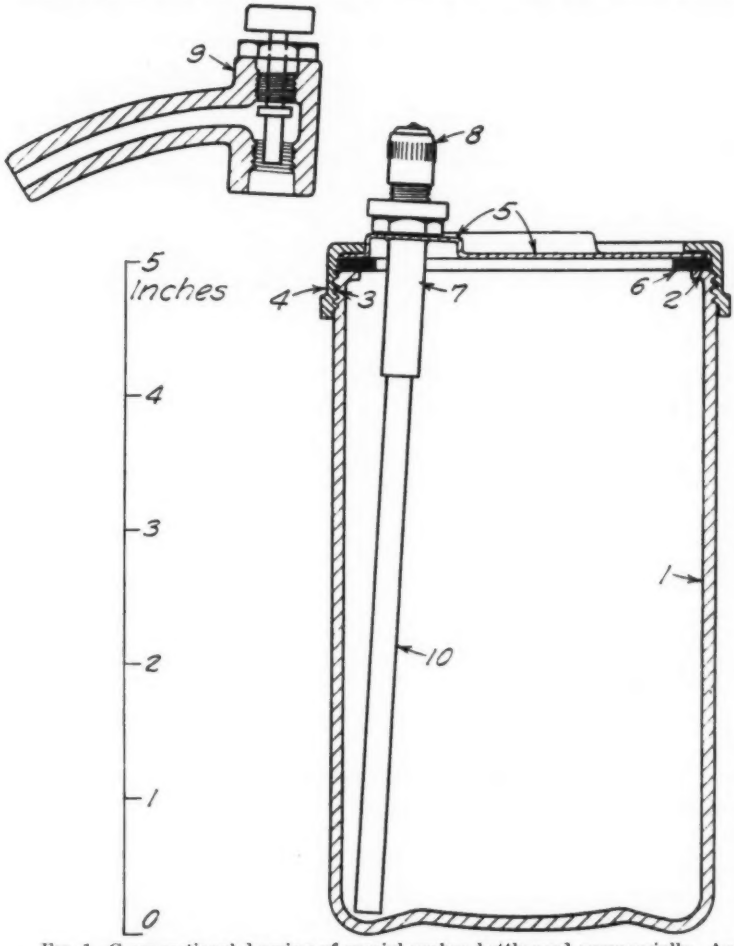


FIG. 1. Cross-sectional drawing of special syphon bottle used commercially. An improved, modified form of dispenser (9) is now available.

and the nitrogen plus air, which was in the bottle, was allowed to rush out. This was repeated three times, thus removing the majority of the air from the overhead gas space and replacing it with nitrogen at atmospheric pressure. The bottle was then placed in a shaking

machine and the valve attached to a nitrous oxide pressure line. The pressure of the gas in the line being regulated by means of a diaphram constant pressure valve unit, the bottle was allowed to shake for 20 seconds, which was the time required for the cream to become saturated at any given pressure. The shaking was merely to increase the rate at which the gas goes into solution in the mix.

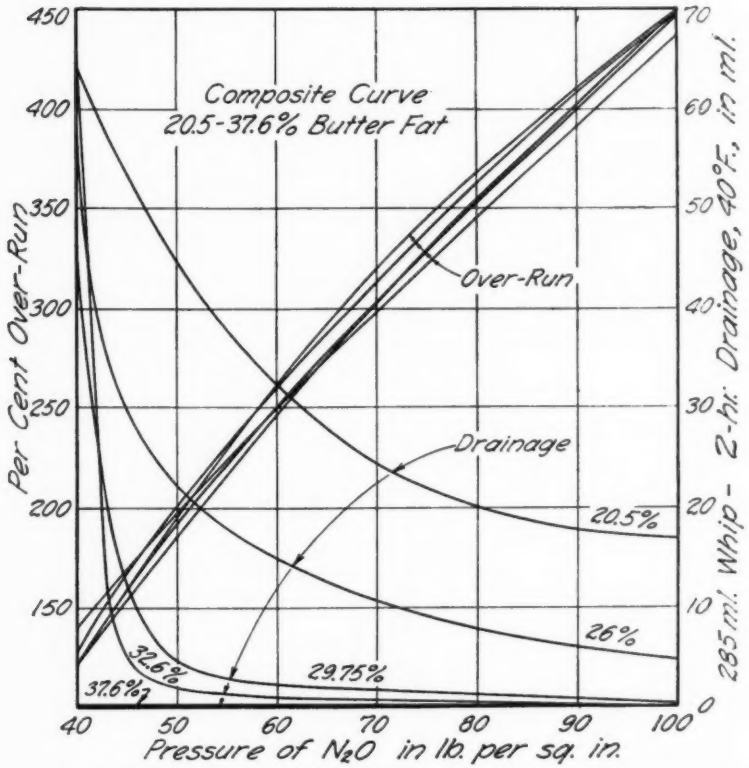


FIG. 2. Relationship of nitrous oxide pressure to overrun and drainage with varying fat content cream-mixes.

The results in the studies were then obtained by allowing the saturated cream-mix to flow into a previously tared 285 ml. tin cup. The whip was cut off level-full with a spatula and the weight of the whip obtained. From this, the percentage overrun was calculated. The 285 ml. of whip was then transferred to a five-inch tin funnel which had a coarse screen fitted across its apex to allow only the thin drainage serum to flow down into a graduated cylinder upon which

the funnel rested. These drainage set-ups were allowed to stand for two hours at 4.4°C.(40°F.) and the amount of drainage was read on the graduations of the cylinder.

Some of the whip was also dispensed in small cups and allowed to stand at room temperature for varying lengths of time in order to observe the appearance and stability of the whip.

RESULTS OF EXPERIMENTS

Relationship of N_2O Pressure to Overrun With Varying Fat Content: A series of studies was carried out in which cream-mixes (mixture of cream, sugar, and vanilla) of varying fat content were saturated at varying pressures of N_2O. Fresh, pasteurized cream from the same plant batch was standardized to 22, 28, 32, 36, and 40 per cent fat content. Each was sweetened with six per cent of sugar and flavored with vanilla. Butterfat analysis was run on each mix thus prepared and the fat content found to be 20.5, 26, 29.75, 32.6, and 37.6 per cent respectively.

TABLE 1

Appearance and Consistency of Whip Immediately After Production

N_2O pressure in pounds per square inch	Percentage of butterfat in the mix [1]				
	20.5	26.0	29.75	32.6	37.6
40	—	—[2]	—[2]	—[2]	+?[2]
45	—	—[2]	—[2]	+[2]	+[2]
50	—	—[2]	+?[2]	+[2]	++[2]
55	—	—[2]	+?[2]	++	+++[2]
60	—	+?[2]	++	+++	+++
65	—	+?	++	+++	+++
70	+?	++	+++	+++	+++
75	+?	++	+++	+++	+++
80	+	+++	+++	+++	+++
85	++	+++	+++	+++	+++
90	++	+++	+++	+++	+++
95	++	+++	+++	+++	+++
100	++	+++	+++	+++	+++

[1] — unsatisfactory; +? barely satisfactory; + satisfactory; ++ good; +++ very good.
[2] These were judged most satisfactory after standing one hour at 26.7°C.(80°F.).

Each of the mixes was studied as follows: A half-pint of the mix was placed in each of 13 bottles and processed as described under "Plan and Procedure of Experimental Work" using N_2O pressures from 40 to 100 pounds per square inch at five-pound intervals. The 65 bottles thus prepared were used to obtain the data as described and presented (Fig. 2 and Table 1).

From the graph, it can be seen that the overrun varies directly with the N_2O pressure and is independent of the butterfat content.

The drainage varies inversely as the butterfat content and N_2O pressure. The table shows that there is a minimum pressure below which the whips were considered unsatisfactory upon production, owing to the fact that they were not very rigid and appeared wet. After the whips stood one hour at room temperature, some of those that were judged unsatisfactory or doubtful at the beginning were the more satisfactory. Since the whip is produced the instant it is to be served, its appearance after standing one hour at room temperature is not considered very significant.

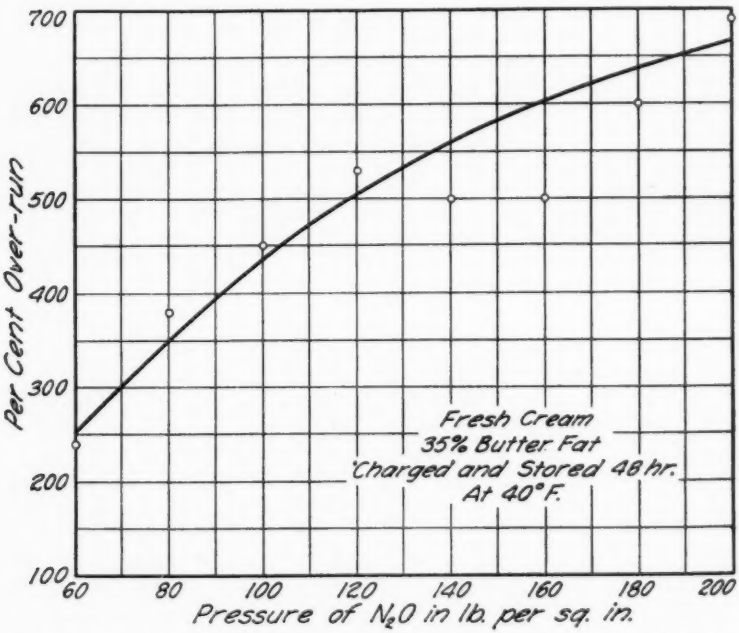
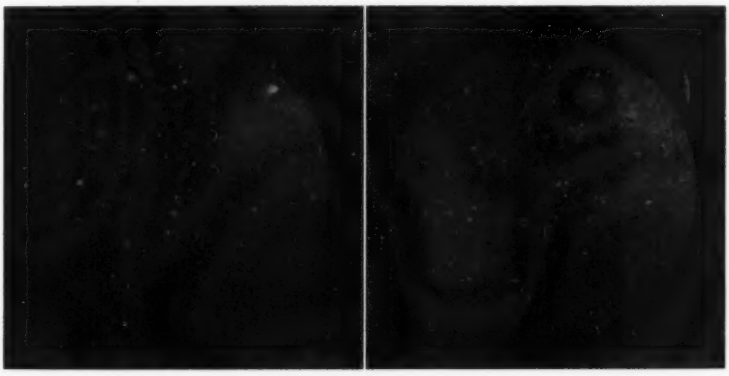


FIG. 3. The effect of increase in nitrous oxide pressure on the overrun obtained.

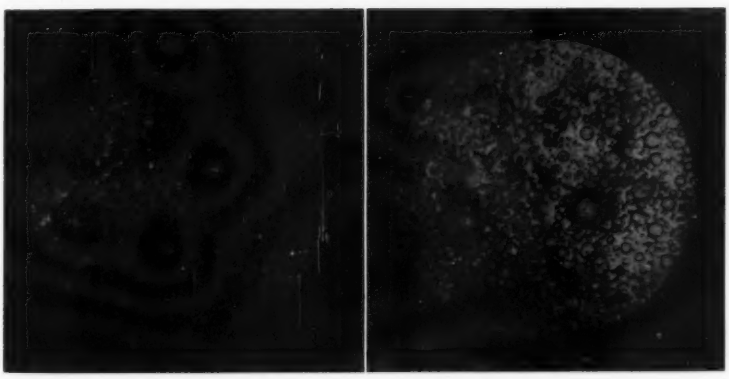
In order to extend the study of the effect on overrun with respect to pressure of N_2O, some of the 32.6-per cent butterfat cream-mix was charged to 120, 140, 160, 180, and 200 pounds per square inch. Here again the overrun continued to increase with an increase in gas pressure. At 200 pounds per square inch N_2O pressure, the overrun exceeded 650 per cent. From the graph (Fig. 3) it can be predicted that even higher overruns could be obtained by saturating at higher pressures than those used. As the gas pressure was increased, it became increasingly difficult to obtain overrun measurements owing to

the fact that the very rigid nature of the whip made it difficult to fill the 285 ml. cup completely without breaking down the whip in attempting to eliminate unfilled pockets in the cup.

Explanation of High Overrun: Since these extremely high overruns would seem unexplainable by present theories regarding whip-



60 lbs./sq. in. N_2O 80 lbs./sq. in. N_2O



150 lbs./sq. in. N_2O Mechanically whipped

FIG. 4. Photomicrographs of the gas cells when whipped by mechanical means and by varying nitrous oxide pressures. (Magnification—30x.)

ping of cream, other studies were made to explain them. Sommer (1935) mentions (a) the efficiency of the whipping device, (b) size of the gas cells, and (c) diameter of the fat globules as being related to the maximum overruns obtainable when whipping cream mixtures.

These three factors are seen to be closely related to maximum overruns obtainable when whipping cream mixtures by the aeration process.

(a) *Efficiency of Whipping Device:* Sommer explains that the efficiency of a whipping device is measured by its ability to beat in or entrap gas without beating out much of the gas already beaten into the cream. Whipping instantly by aeration is extremely efficient as a

TABLE 2

Gas Cells of Various Diameters in Mechanically Whipped and Instantly Whipped Cream

Actual gas-cell diameter	Mechanical whip	Instantly whipped from 40 lbs./sq. in. N_2O	Instantly whipped from 60 lbs./sq. in. N_2O
mm.			
.01	25	1	0
.02	37	15	0
.03	39	48	8
.04	34	69	31
.05	26	44	41
.06	13	21	42
.07	8	5	46
.08	11	1	35
.09	11	0	21
.10	2	0	11
.11	4	0	3
.12	0	0	0
.13	7	0	0
.14	0	0	0
.15	1	0	0
.16	0	0	0
.17	11	0	0
.18	1	0	0
.19	0	0	0
.20	0	0	0
.21	0	0	0
.22	0	0	0
.23	0	0	0
.24	1	0	0
Totals	221	213	238

whipping device because the gas cells are produced within the body of the cream by merely allowing the saturated cream to flow out from under the gas pressure and because there is no mechanical device beating out the gas cells as in mechanical whipping. It has been previously pointed out that larger and larger quantities of gas can be created within the body of the cream by merely increasing the saturation pressure of the gas.

(b) *Size of the Gas Cells:* It is evident that less cream would be necessary to supply laemelle material in a pint of whipped cream if the gas cells were all large than if the gas cells were all small. A study of the diameter of the gas cells was made by use of the microscope on mechanically whipped cream and that whipped by the new process. These studies showed that in the case of mechanically whipped cream the majority of the air cells are about .03 mm. in diameter yet a few cells may be as large as .25 mm. in diameter. The studies of the instant whipped cream show that the gas-cell diameters vary directly with the saturation pressure used before production of the aerated product and are around .10 mm. in diameter. Photomicrographs are

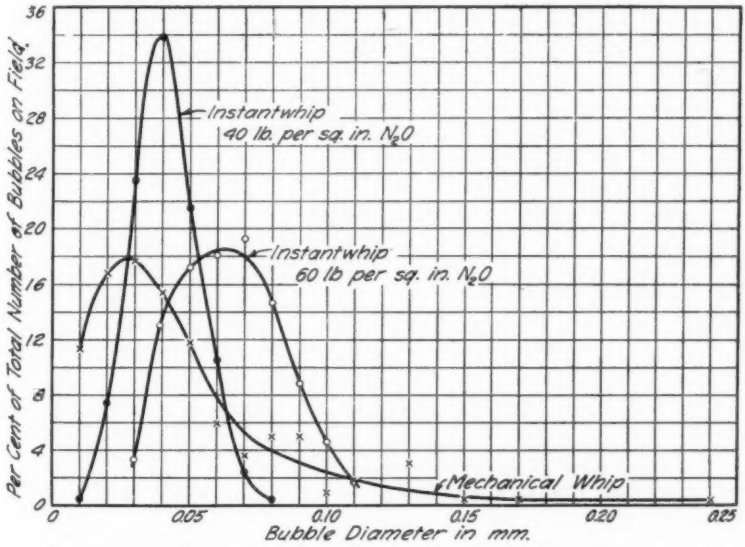


FIG. 5. Comparison of size and number of gas cells with relation to various types of whipping.

shown of the gas cells in mechanically whipped cream and instant whipped cream (Fig. 4). Data were obtained by counting gas cells of the various diameters on other photomicrographs of representative microscopic fields and a graph drawn comparing mechanically whipped cream with instant whipped cream produced from 40 to 60 pounds per square inch N_2O pressure (Table 2 and Fig. 5). As the saturation pressure increases, the diameter of the gas cells increases (Fig. 5). The data in Table 2 were also calculated in terms of volume of the gas cells of the varying diameters (Fig. 6). By comparing the volume of the gas cells with regard to type production, diameter, and percentage

of the total, it can be plainly seen that increase in maximum overrun would be favored by uniformly large gas cells and that this is what instant whipping by aeration tends to produce.

(c) *Decrease in Size of Fat Globules:* The third important factor concerning the degree of maximum overrun is the thickness of the laemelle which is believed to be largely determined by the diameter of the fat globules. The following study was made to determine if

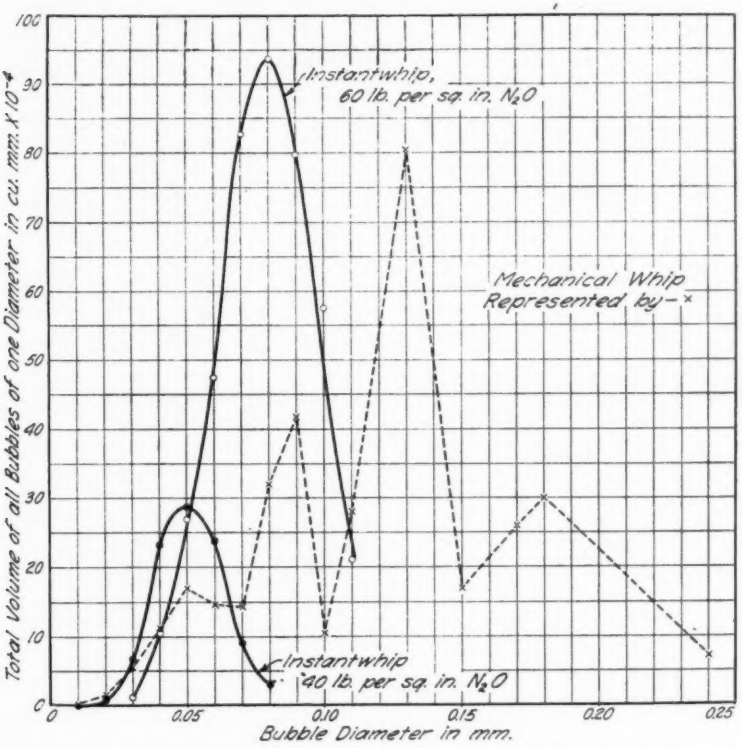


FIG. 6. Total volume of gas cells as a function of gas-cell diameter. (Graphical reproduction of data from Table 2.)

the size of the fat globules was decreased by the instant whipping process.

Ordinarily hemocytometer slides were used with oil immersion objective on the microscope which was fitted with a ruled ocular to allow accurate estimation of the diameters of the fat globules. The ocular rulings were calibrated against a stage micrometer. The diameter of the field was .176 mm. The depth of the hemocytometer slides

was .1 mm. The ocular rulings were equivalent to 1.8 microns on the field. The sizes of the butterfat globules were divided into classes as follows:

Class	Diameter in microns
1	Under 1.8
2	1.8 - 3.6
3	3.6 - 5.4
4	5.4 - up

The studies were made using 35-per cent butterfat cream and N_2O saturation pressure of 80 pounds per square inch.

A weighed portion of the whip was added to a known volume of diluting medium made by mixing one part of water with one part of glycerin as Campbell (1932) suggests. It was found to be impossible to get good resuspension of the whipped product in this medium. Skim milk was used as diluting medium and very good resuspensions were obtained. The skim milk contained large quantities of Class 1 butterfat globules but was free from the large-size classes. One gram of the instant whipped cream was resuspended in 1,000 ml. of this skim milk. Also one gram of the stock cream was added to another portion of the same skim milk for control comparison. Hemocytometer slides were filled and fields were counted of each dilution; the average of nine fields is shown (Table 3).

TABLE 3

Average of Nine Fields

Class size	Average number of globules per field	
	Whipped	Control
2	8.0	27.0
3	1.1	10.0
4	0.7	1.2

When making these counts, no attempt was made to count the Class 1 globules because of the large number present in the diluting medium, but one was impressed by the fact that there were more Class 1 globules in the whipped dilution than in the control dilution.

An attempt was made to dilute these above dilutions with the half water and half glycerine medium and thus be able to count the Class 1 globules. When these secondary dilutions were studied the Class 1 globules had decreased in number per field far greater than the dilution should produce; furthermore, only the larger globules of the Class 1 size were to be seen. Apparently, the difference between the refractive index of butterfat and the glycerine-water medium is not

enough to give the amount of light interference necessary to render the globules visible. Another possibility is that the small globules may actually dissolve in the glycerin-containing medium.

Another secondary dilution was made using water alone as diluting medium, but this dilution allowed such high Brownian movement that the small globules appeared only as dancing points; consequently, focusing and counting was impossible.

A diluting medium consisting of five per cent glycerin, one per cent gelatin, and the rest water was found to give good resuspensions of the whipped product. Direct dilutions of the same concentration were made of the whipped product and of the corresponding control cream. Hemocytometer slides were prepared and ten fields counted (Table 4). The data show that there is a decrease in the number of

TABLE 4

Average of Ten Fields

Class size	Average number of globules per field	
	Whipped	Control
1	24.6	58.0
2	6.7	71.0
3	0.4	45.0
4	0.1	13.0

large butterfat globules. It should be again pointed out that in the Class 1 size the smallest globules that could be focused upon and seen were about one micron in diameter, so it is apparent that the majority but not all of the larger globules are broken down into very small globules.

From study of the efficiency of the whipping device, size of the gas cells, and diameter of the fat globules, it is seen that the overruns obtained by the instant whipping process conform to the accepted theory; however, the process is extremely efficient in overcoming the limiting factors encountered when whipping by mechanical means.

Use of Aged Cream: Studies were carried out using aged cream of varying fat content and varying pressures of N_2O. No specific data are being presented because of the fact that aging did not give reproducible data; however, general trends produced by aging can be given. Other factors remaining the same, aging of cream decreases the overrun that is given. This decrease in overrun is more marked the higher the fat content, which fact seems to indicate that during aging the fat globules undergo some change which alters the ability of the cream to hold the gas that is produced upon dispensing. The

whip from aged cream is rough in appearance and sometimes looks rather shattered.

In general, warming aged cream to 32.2°C.(90°F.) and again cooling it to 4.4°C.(40°F.) will rejuvenate the cream as regards its action when whipped by this process.

Whipping Homogenized Cream: Homogenized cream whips equally as well as ordinary cream by the aeration process. Slightly higher overruns are obtained for any given saturation pressure, but the whip differs some from that obtained from ordinary cream. There is a tendency toward smoother appearance and an increase in the amount of drainage, especially at higher homogenization pressures. The results obtained with cream homogenized at various pressures, as well

TABLE 5

Comparison of Various Cream Treatments Processed at 80 Pounds per Square Inch With N_2O[1]

Type mix	Viscosity [2]	Overrun	Drainage from 285 ml. of whip in 2 hrs. at 40°F.	at once [4] Appearance	Appearance of whip after standing 2 hrs. at 40°F.
		pct.			
Pasteurized.....	12	350	2	++	+
Homo. 200 lbs.[3]...	18	355	1	+++	++
Homo. 400 lbs.[3]...	17	390	2	++++	++++
Homo. 600 lbs.[3]...	16	390	3	+++ (wet)	+
Homo. 800 lbs.[3]...	21	390	7	+++ (wet)	+
.2% gelatin.....	115	300	0	++ (wet)	++
.2% dariloid.....	185	375	0	++++	++++

[1] The butterfat content of all the mixes was 32.5 per cent. [2] These are the degrees of rotation of a No. 26 wire using MacMichael's viscosimeter. [3] Single stage at 65.6°C.(150°F.). [4] + satisfactory; ++ good; +++ very good; ++++ exceptionally good.

as results of pasteurized cream and other treatments to be discussed later, show that although there is more drainage from the homogenized cream-mix whips, their appearance at the time of whipping and two hours later is more satisfactory than that of ordinary pasteurized cream (Table 5).

It should be mentioned that homogenization of the cream decreases the tendency for the fat to rise to the top of the mix upon standing in the bottle, thus reducing the amount of shaking necessary before using in order to insure uniform whip coming from the bottle at all times. The effects of the increased viscosity caused by homogenizing is discussed under "Viscosity of Cream-Mix."

Addition of Foam Stabilizers: Since by the aeration process whipped cream is produced so easily and only in the quantity desired for immediate consumption, there is little need for stabilizing the

whipped product. Some studies were made, however, using gelatin and sodium alginate [1] as additions to the standard 32.5-per cent mix.

Representative data, using two-tenths of one per cent of each substance compared with fresh pasteurized and homogenized cream-mixes, show a marked increase in viscosity of the mix, and also that the gelatin mix does not give as high overruns as the other mixes charged under the same N_2O pressure (Table 5).

Both have beneficial effects on the stability of the whip with regard to drainage and appearance upon standing. There is a tendency for the gelatin mixes to gel to such an extent that all of the mix does not flow out of the bottle upon dispensing. The sodium alginate causes no gel formation.

Viscosity of Cream-Mix: It became apparent during the progressive studies that longer shaking was required to saturate certain mixes at any given N_2O pressure. A study was made to determine how long mixes of different viscosity had to be shaken in order to be sure that they were completely saturated.

Mixes of varying viscosity were prepared by the addition of gelatin, by homogenizing at high pressure, and by the Hening and Dahlberg (1932) method of warming cold cream-mix to 29.4°C.(85°F.) and again cooling it. These various mixes were compared in viscosity by the MacMichael viscosimeter using a No. 26 suspension wire.

In the pint syphon bottles were placed half-pints of the various mixes and the bottles were closed and sealed. They were then swept out with nitrogen as described under "Plan and Procedure of Experimental Work." After replacement of the air with nitrogen, instead of placing the bottles in the shaking machine in contact with a constant N_2O pressure line, they were charged while in the inverted position to 160 pounds per square inch with N_2O without being shaken.

A series of bottles of each mix thus treated were then shaken for varying lengths of time and a measurement of the amount of gas dissolved in each case was made by testing the residual pressure of the gas remaining in the half-pint overhead space. When the pressure ceased to decline with longer shaking, it was assumed that the mix was completely saturated at the pressure of gas in the overhead space. With increased viscosity, longer periods of shaking are required in order to saturate the cream at any given pressure (Fig. 7).

The addition of sodium alginate and gelatin increases the viscosity to such a degree that increased shaking is required to saturate the mix. Homogenization also tends to increase the viscosity and neces-

[1] The commercial product used was Dariloid.

sitates increased shaking, especially when higher homogenization pressures are used.

If the mix is of ordinary degree of viscosity, the shaking machine need not be used in processing with N_2O. The amount of gas needed to saturate the mix at any given pressure and to have the overhead space filled with gas at that pressure is passed into the overhead space while the bottle is in the inverted position. A few vigorous shakes by hand are sufficient to increase the rate of solution so that the mix becomes saturated at the desired pressure.

In the case of the higher viscosity mixes, prolonged shaking is necessary, and for that reason, from a practical point of view, such mixes are not desirable for use in the process.

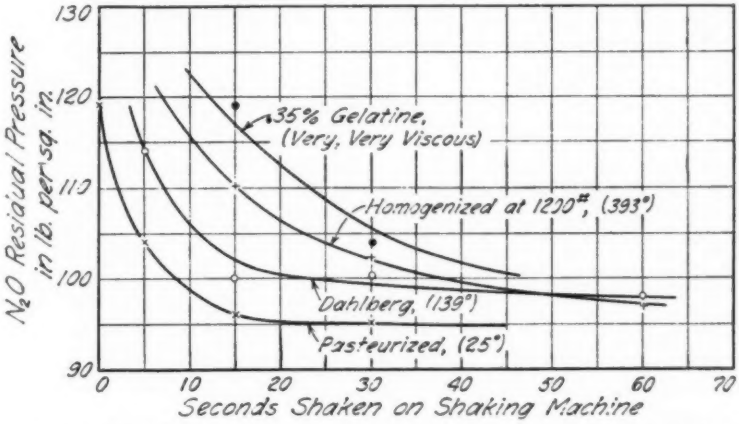


FIG. 7. Variation in required shaking as a function of viscosity to produce saturation. (Numbers in parentheses refer to MacMichael viscosimeter No. 26 suspension wire reading.)

SUMMARY

Whipped cream is produced by aeration by dissolving large quantities of a soluble gas under pressure in liquid cream and then allowing the cream to flow out from under this gas pressure. In a one-pint, all-steel syphon bottle, designed to allow easy cleaning and sterilizing, is placed one-half pint of cream and the bottle is closed. It is then processed by passing nitrous oxide gas (N_2O) into the bottle through the Schrader valve of the syphon tube. Nitrous oxide is used because it is odorless, tasteless, non-toxic, and very soluble.

When the gas is in contact with the cream at one atmosphere pressure (15 pounds per square inch), about 100 volumes of the gas will dissolve in 100 volumes of cream. If the gas pressure is increased

to 30 pounds per square inch, about 200 volumes of the gas at standard conditions will dissolve in 100 volumes of cream. As the pressure of the gas continues to increase, there is a continued proportionate increase in the amount of the gas that dissolves in a fixed amount of cream. The cream remains in the liquid state saturated with the N_2O at elevated pressure as long as it is in the bottle under the gas pressure. When the valve is opened slightly, cream begins to flow out of the bottle, and as it passes through the valve, the pressure drops to ordinary conditions. The gas that was dissolved in the cream owing to the elevated gas pressure comes out of solution in the form of small gas cells uniformly distributed through the body of the cream. As small a quantity of whipped cream as is desired for immediate consumption can thus be produced by simply opening the valve long enough to allow that amount to flow out of the bottle. The cream that remains in the bottle is still in the liquid state and can be continually or intermittently delivered as whipped cream by allowing it to flow out of the bottle. This intermittent production of whipped cream can be continued over a period of a few days or until the bottle becomes empty.

The total volume of whipped cream that is produced from one-half pint of cream by this process varies directly with the pounds per square inch of N_2O pressure to which the cream is saturated. At 200 pounds per square inch, one-half pint of fresh cream will produce three and one-half pints of whipped cream on dispensing. The overrun is independent of the fat content when fresh cream is used. Creams of 20 per cent fat are as easily whipped as is 40-per cent cream. The body of the whip is surprisingly good even at the extremely high overrun of 600 per cent. The stability of the whip upon standing is not as good from the point of view of appearances as is cream perfectly whipped by mechanical means; however, the stability of the whip is of less importance because the whip need not be produced by this process in excess of the amount needed for immediate consumption.

The unusually high overruns that can be obtained by the aeration process are explained by the fact that the process affords a means of entrapping large quantities of gas without the usual beating out of the gas that has already been entrapped. Furthermore, the gas cells are, on an average, larger than those in mechanically whipped cream, thus less cream is necessary to act as laemelle. Besides these two points, the butterfat globules are partially broken down into smaller globules during the whipping process, which allows the formation of thinner laemelle than when larger globules and clumps must be included in the walls of the gas cells.

When aged cream is whipped by this process the overruns are not so high for any given aeration pressure as they are for fresh cream. The tendency toward decreased overruns becomes more apparent as the fat content of the cream increases. Warming aged cream to 32.2 to 35°C.(90 to 95°F.) and cooling it again to 4.4°C. (40°F.) has a rejuvenating effect upon it as regards its whipping properties when whipped by this process.

Homogenized cream whips by this process to give excellent overruns. The quality of the whip is excellent when the homogenization pressure is around 400 pounds. At high homogenization pressure the whip is not as rigid as the whip from ordinary cream even when cream with high fat content is used. At high pressures of homogenization the viscosity of the cream is increased to such a degree that prolonged shaking is required to saturate the cream with the nitrous oxide.

The addition of such substances as gelatin and sodium alginate to increase the stability of the whip are effective. Gelatin is not as satisfactory for use in the cream to be whipped by this process as is the sodium alginate because the cream has a tendency to gel in the bottle. The disadvantage in the use of this type of substance is the increase in the viscosity that it causes. As in the case of viscous homogenized cream, such substances require more shaking to saturate with the gas.

Because of this desire to avoid viscous cream the Dahlberg method of increasing the viscosity of cream does not meet with approval for use on creams to be whipped by this process.

In practical application the process of instantly whipping cream by aeration[1] is as follows: A mix is prepared by adding six per cent of sugar to fresh pasteurized cream of 35 per cent fat content and flavoring it with vanilla. The pressure of nitrous oxide (N_2O) at which the mix is saturated is 80 pounds per square inch. At this pressure seven to eight ounces of mix produce approximately one quart of whip.

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MICROBIOLOGICAL STUDIES ON CULTIVATED BLUEBERRIES IN FROZEN PACK[1]

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(Received for publication, August 2, 1937)

INTRODUCTION

In 1906 the late Dr. Frederick V. Coville,[2] former botanist of the U. S. Department of Agriculture, began his experiments to develop methods for cultivation of the swamp blueberry *(Vaccinium corymbosum)*. These experiments, leading to the production of the large-fruited, hybrid or Coville blueberries now grown under cultivation in many parts of the United States, are well known and were reported by Coville (1921).

Looking forward to the time when the harvest from these plantings will exceed the demands of the fresh fruit market, experiments were carried out to determine the varietal suitability and the packing methods best adapted to the preservation of hybrid blueberries by freezing. This part of the work has been reported by Moon, Smart, and Caldwell (1936).

The microbial content of fresh and frozen blueberries was also studied to determine the effect of freezing storage on the microörganisms normally present on the berries. These studies form the basis of this report.

Diehl, Pentzer, Berry, and Asbury (1934) have described methods of packing and varieties of hybrid blueberries best suited for freezing in the Northwest; but, so far as is known, no work on the microbiological problems connected with the freezing preservation of cultivated blueberries has been reported.

The fruit used in these experiments was donated by the New Jersey Blueberry Cooperation Association through C. S. Beckwith[3] of the New Jersey Agricultural Experiment Station, who is also secretary of the Association. The berries were grown in the vicinity of Pemberton, New Jersey, and consisted of large, fine-quality fruit from the first picking. (Berries of the Cabot variety, which is the

[1] Food Research Division Contribution No. 338.

[2] Dr. Coville, who died Jan. 9, 1937, was greatly interested in the preservation of blueberries by freezing. Many of his suggestions concerning varieties to be tried have been very useful in these studies.

[3] The author wishes to express her grateful acknowledgment of Mr. Beckwith's kindness and coöperation.

earliest to ripen, were from the second picking but were of excellent quality.) Cabot, Concord, Pioneer, Rancocas, and Rubel were the varieties used in these studies. These berries were brought by automobile from Pemberton, New Jersey, to Arlington Experimental Farm, Roslyn, Virginia, where they were held in storage at 0°C. (32°F.) for 18 hours.

PACKING METHODS

The first step in packing the blueberries was to sort them in order to remove any stems, green, crushed, or shriveled fruit, or other débris which might be present. Following this, they were thoroughly washed. Washing the fruit raised the temperature from 0°C.(32°F.) to approximately 12.8°C.(55°F.). The sorting and washing were as carefully done as if the fruit were being prepared for immediate serving. The berries were then weighed into the containers in which they were frozen. Two types of containers were used for the experimental packs: pint paraffined paper cups (a few five-pint paper containers were used) and No. 2 tin cans, having strawberry enamel lining. The berries were packed either in 40, 50, or 60 per cent sugar syrup; in one-third or one-fourth of their weight in dry sugar; or without either syrup or sugar, the latter being known as "dry packs." The filled containers, internal temperature of which was approximately 15.6°C.(60°F.), were then frozen and stored for seven months at —9.4°C.(15°F.).

Cabot, Pioneer, and Concord were selected as having the best flavor and general dessert qualities after preservation by freezing. Rubel and Rancocas varieties were considered inferior to the three varieties just named. Fifty per cent sugar-syrup packs were judged superior to packs prepared in any other way. Some judges considered that more of the flavor and color of the berries was preserved by freezing them in air-tight tin containers, while others thought those packed in non-air-tight paper containers were equally good. Descriptions of varieties, packing methods, and results of the grading will be found in greater detail in the report of Moon, Smart, and Caldwell (1936).

METHODS USED IN MICROBIOLOGICAL STUDIES

The first samples for bacteriological examination were removed after the berries were sorted and washed. Samples consisted of 100 grams selected from various parts of the mass in order that the sample be as representative as possible. To the 100 grams of berries, which were weighed into a sterile jar, were added 100 c.c. of sterile tap water. The berries were mashed and mixed thoroughly with the water, after which proper dilutions were made and plates poured.

Two different kinds of media were used in these studies: beef infusion agar adjusted to pH 7.0 and wort agar with a pH value near 4.5. The beef agar supported a good growth of bacteria while the wort agar was found to be especially good for the growth of yeasts and molds. The beef agar plates were incubated two days at 30°C.(86°F.), and the wort agar plates were incubated for five days to allow the molds to sporulate.

At the end of the incubation period the colonies occurring on the plates were counted. The predominating types of microörganisms were then selected and run through the usual tests for identification.

MICROBIAL CONTENT OF FRESH CULTIVATED BLUEBERRIES

The blueberries used in this experiment were gathered from plants which were practically free from bacterial or fungous diseases. The

TABLE 1

Average Microbial Content of Fresh and Frozen Cultivated Blueberries

Variety	Fresh berries (washed)—Average number microörganisms	Frozen berries—Average number microörganisms	Reduction microbial content after 7 months at —9.4°C.(15°F.)
	per gm.	*per gm.*	*pct.*
Cabot	1,200,000	1,500	99.9
Concord	155,000	1,000	99.4
Pioneer	160,000	800	99.5
Rancocas	100,000	500	99.5
Rubel	250,000	1,000	99.6

only types of microörganisms isolated from the fresh berries were the types ordinarily found in soil and water, which are very common sources of contamination. The numbers of bacteria, yeasts, and molds on fresh blueberries as well as the numbers found on the frozen fruit are given (Table 1), as are the names of the microörganisms repeatedly isolated from fresh and frozen blueberries (Table 2).

The microbial content of the varieties of blueberries listed, with the exception of Cabot, are fairly low as compared with that of other fresh fruits and vegetables. The higher microbial content of the Cabot berries may have been due to the characteristics of bushes of this variety. Cabot bushes are low and spreading and consequently the fruit is borne nearer the soil. Any rains falling just before the berries are picked would be likely to spatter them considerably more than would be the case on higher and more compact bushes.

Cabot variety berries were of the second picking and it may have been that a few overripe berries were left on the bushes. This overripe fruit was not included in the frozen packs, but it may have served to contaminate the sound fruit before it was picked.

The proportion of yeasts and molds to bacteria present on the fruit was also greater in the Cabot variety than was the case with other varieties. These types may have been dust-borne or insect-borne. The contamination from the latter may have been due to the great attraction that the few overripe berries, which may have remained on the bushes after the first picking, have for insects. Berries of the Cabot

TABLE 2

Microörganisms Repeatedly Isolated From Fresh and Frozen Cultivated Blueberries

Fresh blueberries	Frozen blueberries
Bacteria:	Bacteria:
Aerobacter aerogenes (Kruse) Beijerinek	*Bacillus albolactis* Migula
Bacillus albolactis Migula	*Bacillus cereus var. fluorescens.* Laubach
Bacillus cereus var. fluorescens Laubach	*Bacillus megatherium* DeBary
Bacillus mycoides Flügge	*Bacillus mycoides* Flügge
Bacillus simplex Gottheil	*Bacillus subtilis* Cohn *emend* Prazmowski
Flavobacterium sp.	
Pseudomonas fluorescens Migula	
Yeasts:	Yeasts:
Mycoderma sp.	*Mycoderma* sp.
Saccharomyces sp. (pink, white, and black species)	*Saccharomyces* sp. (pink, white, and black species)
Schizosaccharomyces sp.	
Molds:	Molds:
Botrytis sp.	*Monilia* sp.
Cladosporium sp.	*Penicillium* sp.
Monilia sp.	*Rhizopus* sp.
Penicillium sp.	
Rhizopus sp.	
Sclerotinia sp.	

variety, in spite of the higher microbial content, were of excellent quality and there was no loss of fresh appearance and no softening in texture.

Great care should be taken in the handling of blueberries to keep them cool until packed and to handle and freeze them as rapidly as possible to avoid spoilage.

MICROBIAL CONTENT OF FROZEN BLUEBERRIES

While the microbial content of fresh cultivated blueberries is reduced over 99 per cent (Table 1) by storing them at freezing tem-

peratures for seven months, there are a sufficient number of bacteria, yeasts, and molds remaining alive in the frozen berries to cause spoilage in the thawed berries in a short while at room temperature. The frozen berries should be utilized as soon as thawed, or stored in a refrigerator until used.

The types of microörganisms surviving the storage period in frozen blueberries are those ordinarily found on fresh fruit and have no effect on the healthfulness of the product provided the fruit is eaten soon after defrosting. One or two types of bacteria appear in the list (Table 2) of organisms isolated from the frozen berries which do not appear on the list of those present on the fresh fruit. These types may have been introduced with the syrup or from the utensils or containers used in putting up the packs.

Where the fruit is of good quality and the packing and freezing are carried out without delay, cultivated blueberries in frozen pack seem to offer few problems of a microbiological nature.

SUMMARY

Five varieties of cultivated blueberries grown in New Jersey were used in preparing experimental frozen packs of this fruit to determine the packing methods best suited to the retention of fresh-fruit quality and also to determine the possible relationship between the microbial content of fresh and frozen blueberries and the quality of the frozen product.

Moon, Smart, and Caldwell (1936) have reported on the results of the study of packing methods.

The microbial content of fresh cultivated blueberries varies in numbers from 100,000 to over a million per gram. Soil and water types of microörganisms are the predominating kinds isolated from the fresh berries.

While less than one per cent of the microörganisms survived the storage period of seven months in the frozen berries, the numbers and types remaining alive in the fruit are sufficient to cause spoilage in a short while at room temperature.

The microbial types present in the frozen berries in no way affect the healthfulness of the product provided the fruit is consumed promptly after defrosting.

When cultivated blueberries of good quality are washed, packed, and frozen without undue delay, the microbial content of the frozen product should offer no problems to the packer or consumer.

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BACTERICIDAL EFFECTS OF ONION VAPORS

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(Received for publication, August 13, 1937)

Walton, Herbold, and Lindegren (1936) reported that garlic vapors have a bactericidal effect. Onion vapors are also bactericidal but to a lesser extent.

EXPERIMENTAL PROCEDURE

One gram of succulent onion scales, finely ground in a meat grinder, was placed in the tops of inverted Petri dishes, the bottoms of which contained 15 c.c. of two-per cent glycerine agar. The Petri

TABLE 1

Amount of Growth on Plates After Exposure to Onion Fumes

	Control	32 min.	1 hr.	2 hr.	4 hr.
Bacillus subtilis 24-hr. culture	4	4	3	2	1
	4	3	3	1	1
	4	3	3	1	0
	4	3	2	1	0
Bacillus mycoides 24-hr. culture	4	4	3	2	1
	4	4	3	2	0
	4	3	3	2	0
	4	3	2	2	0
Serratia marcescens 24-hr. culture	4	4	4	4	4
	4	4	4	4	3
	4	4	4	4	3
	4	3	4	4	3
Mycobacterium smegmatis 72-hr. culture	4	3	2	1	0
	4	2	2	1	0
	4	2	2	1	0
	4	2	1	0	0

dishes were sealed and placed at 37°C.(98.6°F.) in an incubator for various lengths of time. After exposure of the agar to the onion fumes the covers with the onion in them were removed, the agar streaked with the suspension of a given organism, clean empty tops put on, and the dishes resealed and incubated at 37°C. Controls were used in all experiments. In the comparative scale used in the charts, 4 indicates full growth (such as found on the controls), 3 indicates between three-quarters and full growth, 2 indicates between

one-half and three-quarters of full growth, and 1 indicates any growth at all to one-half of full growth.

Tests were made with *Serratia marcescens, Bacillus subtilis, Bacillus mycoides,* and *Mycobacterium smegmatis* on agar exposed from 32 minutes to four hours (Table 1).

The order of increasing resistance to the vapors of onion is *Mycobacterium smegmatis, Bacillus mycoides, Bacillus subtilis,* and *Serratia marcescens.* Onion vapors are not as bactericidal as those of garlic according to Walton, Herbold, and Lindegren. The acid-fast organism, *Mycobacterium smegmatis,* was the least resistant. *Serratia marcescens* was the most resistant, even more resistant than *Bacillus subtilis.*

TABLE 2

Toxicity of Onion Vapors at Incubator, Room, and Ice-Box Temperatures
(Each number represents an average of four determinations)

		Control	32 min.	1 hr.	2 hr.	4 hr.
Bacillus mycoides	Incubator	4	3.50	2.75	1.50	0
	Room	4	3.25	3.50	1.25	0
	Ice-box	4	3.75	2.75	2.25	2.25
Bacillus subtilis	Incubator	4	3.25	2.75	1.25	0
	Room	4	3.25	2.75	3.25	2.00
	Ice-box	4	4.00	4.00	4.00	4.00
Mycobacterium smegmatis	Incubator	4	2.50	1.75	1.50	0
	Room	4	3.00	2.00	2.00	1.75
	Ice-box	4	3.00	2.75	2.50	1.50
Serratia marcescens	Incubator	4	3.75	4.00	4.00	3.00
	Room	4	4.00	4.00	4.00	4.00
	Ice-box	4	4.00	4.00	4.00	4.00

With the same organisms a comparison was made of the effects of the fumes from one gram of onion at incubator, room, and ice-box temperatures. These tests indicate that more onion vapors are given off at 37°C. than at 18°C.(64.4°F.) and more at 18°C. than at 10°C. (50°F.) (Table 2).

A comparison of the resistance of the spores and vegetative forms of *Bacillus subtilis* and *Bacillus mycoides* to onion vapors showed that spores of the organisms are only slightly more resistant than the vegetative forms (Table 3).

Tests were made to determine time required to exhaust the bactericidal substances emitted from one gram of freshly ground onion. An agar plate was exposed for four hours to one gram of freshly ground onion. After four hours the plate was streaked, a clean cover put on, and a second plate was placed over the original one gram of

onion. These same steps were then repeated after the second four-hour period. The results (Table 4) indicate that the bactericidal effect of the onion vapors is more quickly exhausted than that of garlic (Walton, Herbold, and Lindegren).

TABLE 3

Comparison of Onion Vapors on Spores and Vegetative Forms of Bacillus subtilis and Bacillus mycoides

(Each number represents an average of four determinations)

		Control	32 min.	1 hr.	2 hr.	4 hr.
Bacillus subtilis spores	2-month culture	4	2.75	2.25	1.75	1
Bacillus subtilis vegetative	24-hour culture	4	3.25	2.75	1.25	0
Bacillus mycoides spores	2-month culture	4	3.75	3.00	0.25	0
Bacillus mycoides vegetative	24-hour culture	4	3.50	2.75	0	0

DISCUSSION

The bactericide in onion is different from that in garlic. Onion loses its bactericide after grinding much more quickly than does garlic. Garlic may be ground and left in the open air for one or

TABLE 4

Exhaustion of Fumes From One Gram of Onion

	Control	1st 4-hr. period	2nd 4-hr. period	3rd 4-hr. period
Bacillus subtilis	4	0	4	4
	4	0	4	4
	....	0	4	4
	....	0	4	3

two hours without appreciable loss in bactericidal vapors; ground onion loses a considerable amount in ten minutes. This makes it difficult to obtain standard results in tests. The onions used throughout these experiments were small, white boiling onions, the strongest available on the market at the time the experiments were made.

CONCLUSIONS

1. Onion vapors have a bactericidal effect.

2. The bactericidal emissions from one gram of onion increase with an increase in temperature from 10°C. to 37°C. and are exhausted by the successive exposures of a given amount of onion to agar plates.

3. The bactericidal effect of onion vapors is not as strong as, and is more quickly exhausted than, that of garlic vapors when tested under like conditions.

ACKNOWLEDGMENT

The author is grateful for the assistance of Dr. Carl C. Lindegren throughout the study of this problem.

REFERENCE

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CORROSION OF METALS BY MUSTS AND WINES

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(Received for publication, August 13, 1937)

Since the repeal of prohibition and subsequent revival of the wine industry, many different metals and alloys have been included in winery equipment used in contact with musts and wines. Relatively little information exists on the behavior of these metals when subjected to various conditions existing at different stages of wine making. Searle, La Que, and Dohrow (1934) conducted an extensive series of corrosion tests in an eastern winery. The grapes and methods used in the wine industry of eastern North America, however, are very different from those used in California, so their data cannot be applied to the California wine industry. In order to obtain corrosion data under California conditions, tests were conducted with 46 metals and alloys in three California wineries.

EXPERIMENTAL PROCEDURE

The methods used were similar to those employed by Searle, La Que, and Dohrow. Tests were conducted in two wineries located at Lodi where large wineries predominate and one in the Livermore valley where the wineries are smaller and devoted to the production of dry wines. Crusher, sump, and fermenter tests were conducted in the Livermore winery and in one Lodi winery. All other tests were conducted in one or both of the Lodi wineries.

The metals tested were similar to those commonly used or suggested for use in winery equipment. An attempt was made to select metals representing as wide a range of properties as possible. In all instances test pieces were cut to a surface area of approximately four square inches, determined accurately for each piece by the use of micrometer calipers. Some of the stainless steels were cut and then welded together in order to determine the effect of welding on the resistance to corrosion. A list of metals used and their approximate compositions are given (Table 1).

The test conditions were those actually existing in the wineries. As the conditions in any particular operation usually varied during a given test period, an effort was made to obtain an accurate log of the changes during each period. This information was often difficult to

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TABLE 1

Composition of Metals and Alloys Tested

(Values given in percentages)

Code No.	Metal	Cu.	Sn.	Fe.	Ni.	Al.	Pb.	Si.	Zn.	Mn.	Cr.	C.	P.	Mg.
LB	Pure al.	....	.....	.....	.....	99.0	....	....	....	....	....	....	....	....
86	Pure al. 86	....	.....	.....	.....	99.5+	....	....	....	....	....	....	....	....
55	Al. alloy	....	.....	.....	.....	98.5	....	....	....	....	.25	....	....	1.25
12	Al. alloy 12	6	.....	.....	.....	92.0	....	2	....	....	....	....	....	....
76	Al. alloy 76	....	.....	.....	2	98.0	....	....	....	....	....	....	....	....
13	Al. bronze	89	.....	1	.....	10.0	....	....	....	....	....	....	....	....
18	Acid bronze	88	10	.....	.....	.....	2	....	....	....	....	....	....	....
19	Cupro-nickel	60	.....	.....	40	.....	....	....	....	....	....	....	....	....
11	Everdur	95	.....	.....	.....	.....	....	4	....	1	....	....	....	....
16	Machine brass	85	5	.....	.....	.....	5	....	5	....	....	....	....	....
15	Manganese bronze	58	0.75	0.9	.....	0.75	....	....	39.6	.06	....	....	....	....
14	Phosphorous bronze	90	10.00	.....	.....	.....	....	....	....	....	....	....	+	....
10	Waukesha alloy	60-63	.....	9-10	28-30	.....	....	....	....	....	....	....	....	....
NC	Inconel	....	.....	6	80	.....	....	....	....	....	13	....	....	....
33	Niresist	6	.....	78 cast iron	14	.....	....	....	....	....	2	....	....	....
M	Monel metal	29	.....	1.5	68	.....	....	....	....	....	....	....	....	....
C	Copper	99.9	.....	.....	.....	.....	....	....	....	....	....	....	....	....
N	Nickel	....	.....	.....	99.5	.....	....	....	....	....	....	....	....	....
22	Silver (pure)[1]	....	.....	.....	.....	.....	....	....	....	....	....	....	....	....
21	Tin (pure)	....	99.9	.....	.....	.....	....	....	....	....	....	....	....	.
UL	18-8 Alloy	....	.....	74	8	.....	....	....	....	....	18	....	....	....
17	Cast iron	....	.....	(Difference)	.....	.....	....	1.75	....	.6	....	3	.15-.3	....
20	Steel	....	.....	(Difference)	.....	.....	....	....	....	.3-.6	....	.1-.2	....	....

[1] Commercial grade analyses unknown.

TABLE 1 *(Concluded)*

Code No.	Metal	Fe.	Ni.	Cr.	C.	Mo.	Ti.
111	Allegheny C (pickled finish)[2]	.....	.....	.....	.....	.....	.....
44	Allegheny C (cold rolled)[2]	.....	.....	.....	.....	.....	.....
N2795	Durimetl[2]	.....	.....	.....	.....	.....	.....
S1	Stainless steel (type 501 c)	(Difference)	.....	4-6	.11-.15	.....	.....
S2	Stainless steel (type 302)	(Difference)	7-9	17-19	.08-.2	.....	.....
S3	Stainless steel (type 302 welded)	(Difference)	7-9	17-19	.08-.2	.....	.....
S4	Stainless steel (type 304)	(Difference)	7-9	17-19	Max. .11	.....	.....
S5	Stainless steel (type 304 welded)	(Difference)	7-9	17-19	Max. .11	.....	.....
S6	Stainless steel (type 306)	(Difference)	8-10	18-20	Max. .11	.....	.....
S7	Stainless steel (type 309)	(Difference)	11-13	22-26	Max. .20	.....	.....
S8	Stainless steel (type 316)	(Difference)	7-11	17-19	Max. .11	2-4	.....
S9	Stainless steel (type 321)	(Difference)	8-10	18-20	Max. .20	.....	4 times C
S10	Stainless steel (type 321 welded)	(Difference)	8-10	18-20	Max. .20	.....	4 times C
S11	Stainless steel (type 410)	(Difference)	.....	12-14	Max. .12	.....	.....
S12	Stainless steel (type 430)	(Difference)	.....	16-18	Max. .12	.....	.....
S13	Stainless steel (type 446)	(Difference)	.....	23-30	Max. .35	.....	.....
110	Metallized aluminum[3]	.....	.....	.....	.....	.....	.....
120	Metallized tin[3]	.....	.....	.....	.....	.....	.....
130	Metallized copper[3]	.....	.....	.....	.....	.....	.....
56	Nickel plate[3]	.....	.....	.....	.....	.....	.....
57	Tin plate[3]	.....	.....	.....	.....	.....	.....
58	Chromium plate[3]	.....	.....	.....	.....	.....	.....
59	Silver plate[3]	.....	.....	.....	.....	.....	.....

[2] Composition unknown for samples used. [3] Composition unknown.

obtain, however, and when an accurate log was secured, it was practically impossible to duplicate the conditions exactly for other tests. In view of the circumstances, the metals were usually exposed at each point for comparatively long periods of time ranging from three to seven days. By using these long test periods the metals were exposed to an average representative set of conditions for the test point concerned. All tests, except those with the metallized metals, were conducted in duplicate. Test metal samples were exposed at the following places in the wineries: crushers, sumps, fermentation vats, storage tanks, sherry cookers, and filter chargers.

Crusher tests were conducted for about 12-hour periods because crushing was normally a daytime operation and at the end of each day the crushers were washed thoroughly with water. The 12-hour exposure was considered sufficient because in most instances corrosion was relatively heavy in the crusher tests. Test spools of metals were suspended about 12 to 18 inches beneath the crusher where the freshly crushed grapes and must continuously passed over the metals as long as the crushing operation was in progress. This type of exposure favored intermittent immersion and aeration.

Spools of metals exposed during fermentation were suspended under the caps about two feet beneath the surfaces of 2,000- and 10,000-gallon fermentation vats having six to eight feet of must. Exposures were made near the surface because, according to Searle, *et al.*, the most intensive corrosion occurs near the surface. The length of exposure was usually for the duration of the period of fermentation, although in a few instances the test samples remained in the must during two or three successive fermentations. This was the case when the fermentation period was relatively short as in the preparation of certain fortified wines. The grapes used in all fermentations were mixtures of several vinifera varieties of red or white grapes. Yeast starters were added to all vats of must. Although SO_2 was usually added to fresh must before the start of fermentation, tests were made in musts containing no added sulfur dioxide as well as in musts containing sulfur dioxide. When SO_2 was used, it was always added to freshly crushed must in the form of potassium metabisulfite. Sufficient potassium metabisulfite was added to give the must a concentration of from 75 to 100 parts per million of sulfur dioxide. The temperatures during fermentation ranged from 17.78 to 30.56°C.(64 to 87°F.) although in most instances it was about 23.89°C.(75°F.). The musts in all fermenting vats were circulated (pumped over) at least once during the fermentation period.

In tests conducted in sumps, spools of metals were placed on the sump floor and allowed to remain there several days. The type (red

or white) and quantity of wine that flowed through the sump during the test period varied intermittently. The wines dropped about two feet through the air upon entering the sumps; consequently they were partially aerated when in contact with the metals.

Spools of metals were also exposed in metal filter chargers of about 150-gallon capacity, near the edge of the tank, midway between top and bottom, and on the side of the tank opposite the agitators. The metals were continuously in the path of the moving wine and particles of filter aid. All tests were made at room temperature. Aeration of the wine caused by constant agitation and the abrasive action of the filter aids were undoubtedly responsible for some of the heavy losses in weight of test samples observed in the filter charger.

Exposures were also made in storage tanks containing port, sherry stock, Zinfandel, white blend, Sauterne, and Riesling type wines. All wines were new and of the current season's vintage. The storage tanks were about 20 feet deep and of 30,000- to 40,000-gallon capacity. The temperatures of the stored wines ranged from 21.11 to 26.67°C. (70 to 80°F.). The metals were suspended about two feet beneath the surface and near the center of the tanks.

Sherry cooker tests were made in 40,000-gallon tanks in a manner similar to that used in the storage-tank tests. Exposures were made at 43.33°C.(110°F.) and at 57.22°C.(135°F.). In the latter case the wine was not circulated, whereas the wine heated to 43.33°C. was circulated slowly. The sherry-cooker exposures were longer than most of the other tests because it was necessary to leave the metals in the tanks for the duration of the heating periods which ranged from seven to 15 days.

The data obtained are recorded in milligrams of loss per square decimeter of surface per day ($mg/dm^2/d$) rather than in inches penetration per year since the factor of metallic contamination of wines is of greater importance to the wine industry than is the loss of equipment caused by corrosion. The data given are the averages of two or more tests, the results of which usually agreed within 10 per cent, except when the losses were slight as in the case of the stainless steels or when the losses were extremely heavy as with cast iron and steel (Table 2).

EXPERIMENTAL RESULTS

Losses of less than .1 $mg/dm^2/d$ were considered insignificant and test pieces that consistently showed such small losses are not included (Table 2) but are considered in the discussion.

Resistance of Various Metals to Corrosion: The amount of corrosion observed varied considerably with the type of metal or alloy used.

TABLE 2

Average Losses in Weight of Test Pieces During Various Wine-Making Operations

(Given in milligrams per square decimeter per day)

Code No.	Metal	White crusher	Red crusher	White sump	Red sump	White fermenter	White ferm.+SO_2	Red fermenter	Red ferm.+SO_2
LB	Alum. pure	0.7	1.2	0.5	0.2	2.5	0.5	0.0	3.3
86	Alum. No. 86	13.6	27.2	.....	.....	.....	.....	.....	.....
55	Alum. alloy No. 55	3.8	23.6	.....	.....	.....	.....	.....	.....
12	Alum. alloy No. 12	19.4	1.3	1.0	0.4	.....	1.3	1.4	0.5
76	Alum. alloy No. 76	1.7	1.7	0.6	0.1	1.4	0.1	0.2	1.0
13	Alum. bronze	4.2	0.2	1.2	2.7	0.0	0.0	0.0	0.6
18	Acid bronze	9.6	1.1	1.9	1.0	0.0	1.0	1.0	0.3
19	Cupro-nickel	0.0	0.0	0.6	1.4	0.5	0.0	0.0	1.4
11	Everdur	12.4	1.4	1.9	1.6	0.4	0.3	0.1	0.2
16	Machine brass	42.3	38.0	5.1	1.9	0.6	12.4	11.9	0.4
15	Manganese bronze	12.7	4.3	2.3	1.8	0.2	2.8	0.8	0.1
14	Phosphorous bronze	15.5	3.9	1.2	1.5	0.0	0.0	0.1	0.0
10	Waukesha alloy	8.6	2.0	0.0	0.6	1.5	0.0	0.2	3.6
NC	Inconel	0.0	1.2	0.0	0.1	0.1	0.2	0.2	0.1
33	Niresist	.....	.....	.....	.....	.....	.....	.....	.....
M	Monel metal	11.8	32.0	2.0	9.1	0.3	0.7	0.5	0.5
N	Nickel	40.3	63.4	7.8	5.6	1.4	3.2	0.9	5.4
C	Copper	24.5	1.8	2.7	1.6	0.6	1.8	1.0	0.2
21	Tin	22.1	21.2	4.0	0.3	2.9	89.6	17.5	15.2
22	Silver	7.0	24.3	.....	.....	.....	.....	.....	.....
UL	18-8 Alloy	0.0	0.6	0.0	0.0	0.0	0.0	0.2	0.4
17	Cast iron	305.3	238.5	143.3	54.6	149.4	208.5	118.1	148.7
20	Steel	281.1	216.8	41.3	30.5	10.5	72.8	35.5	24.6
56	Nickel-plated cu	78.0	94.5	0.3	.....	1.3	.....	0.8	.....
57	Tin-plated cu	10.2	8.2	1.8	.....	3.3	.....	12.2	.....
58	Chromium-plated cu	5.0	5.0	0.1	.....	0.2	.....	0.1	.....
59	Silver-plated cu	0.0	31.8	0.1	.....	0.1	.....	1.0	.....

Code No.	Metal	Port storage	Sherry stock	New dry red	New dry white	Sauterne storage	Filter charger	Sherry cooker 43.33°C. (110°F.)	Sherry cooker 56.67°C. (135°F.)
59	Silver-plated cu	0.0	31.8	0.1	.....	0.1	.....	1.9	.....

TABLE 2 *(Concluded)*

Code No.	Metal	Port storage	Sherry stock	New dry red	New dry white	Sauterne storage	Filter charger	Sherry cooker 43.33°C. (110°F.)	Sherry cooker 56.67°C. (135°F.)
LB	Alum. pure	1.9	1.6	2.9	3.9	2.5	1.4	1.9	1.8
86	Alum. No. 86	2.2	0.6	.....	0.2	.....	3.6	0.0	1.2
55	Alum. alloy No. 55	2.2	0.7	.....	1.4	.....	0.9	4.3	1.9
12	Alum. alloy No. 12	0.0	0.2	1.3	1.4	0.0	1.6	0.0	0.7
76	Alum. alloy No. 76	1.4	0.9	1.7	2.4	1.0	1.1	0.3	1.1
13	Alum. bronze	0.1	1.2	0.7	0.9	2.0	2.5	1.8	0.0
18	Acid bronze	0.3	1.3	0.7	0.7	2.9	3.5	0.5	0.0
19	Cupro-nickel	0.0	1.2	0.0	0.4	5.6	0.8	0.4	0.0
11	Everdur	0.0	1.1	1.3	0.4	3.8	2.1	0.7	0.1
16	Machine brass	1.1	2.3	2.7	2.8	4.4	3.1	0.9	0.4
15	Manganese bronze	1.4	1.7	1.2	2.2	4.8	4.0	0.8	0.1
14	Phosphorous bronze	0.1	1.2	0.9	1.8	2.6	2.0	0.7	0.1
10	Waukesha alloy	0.0	0.8	0.7	1.6	4.0	1.6	0.0	0.0
NC	Inconel	0.0	0.1	0.1	0.2	0.0	0.0	0.0	0.0
33	Niresist	.....	4.9	.....	.....	46.8	226.0	12.8	39.2
M	Monel metal	0.2	2.7	0.0	0.7	9.2	4.1	1.2	0.1
N	Nickel	1.6	15.9	2.2	1.4	25.6	241.5	6.7	2.1
C	Copper	0.8	1.4	1.2	1.5	4.4	2.2	1.0	0.0
21	Tin	46.0	0.3	90.0	6.0	0.3	123.5	0.2	10.3
22	Silver	.....	0.0	.....	0.8	.....	.....	0.6	0.6
UL	18-8 Alloy	0.2	0.2	0.2	0.3	0.0	0.0	0.0	0.0
17	Cast iron	.....	.....	.....	.....	.....	.....	.....	.....
20	Steel	.....	.....	.....	.....	.....	.....	.....	.....
56	Nickel-plated cu	.....	4.0	1.0	.....	.....	23.6	.....	.....
57	Tin-plated cu	.....	17.9	8.5	.....	.....	0.5	.....	.....
58	Chromium-plated cu	.....	0.6	0.1	.....	.....	0.3	.....	.....
59	Silver-plated cu	.....	1.1	0.0	.....	.....	0.6	.....	.....

Stainless steels S1, S13, 44, 111, and UL, which were exposed to all test conditions used, in nearly all instances lost less than .1 mg/dm²/d and in most instances less than .05 mg/dm²/d. Stainless steel UL was corroded more heavily than the other steel samples and for this reason its losses are included (Table 2). In nine of the 16 winery operations where these steels were exposed, the losses were so slight that they could not be detected. In all other tests the losses were slight. All samples of stainless steels retained the original surface appearance and color after prolonged exposure. In actual winery practice it has been observed that stainless steel equipment sometimes corrodes rapidly where welded. The welding procedure has been improved, however, and this type of corrosion is no longer common. Samples S3, S5, and S10 welded by the improved technique did not corrode any faster than the corresponding unwelded samples. The surface condition of the stainless steels did not perceptibly affect their resistance to corrosion since smooth, cold-rolled and pickled-finished samples of Allegheny-C were equally resistant to corrosion. Metals with rough, irregular surfaces tended to accumulate firm deposits of tartrate which seemed to serve as a protective coating. Durimetl-N2795 and metallized metals 110, 120, and 130 had very irregular surfaces and consequently accumulated on their surfaces considerable quantities of tartrates which were very difficult to remove. For this reason dependable data on the corrosion of these metals was not obtained; but it appeared that as long as the tartrate coating covered these metals they were protected from severe corrosion.

Corrosion data obtained for the other metals and alloys tested show that the losses of most of these metals varied considerably with the particular place of exposure (Table 2). The magnitude of losses ranged from zero in several instances to over 300 mg/dm²/d for cast iron exposed at the white-grape crusher. The weight-loss distribution of all metals under all test conditions indicates that the most resistant metals tested, in addition to the stainless steels, were 18-8 alloy, Inconel, and Aluminum-alloy 76 (Table 3). The metallized metals and Durimetl also fall into this group, but as indicated above, the actual losses caused by corrosion were not obtained because of tartrate deposition. A second group of relatively resistant metals consisted of aluminum, copper, Aluminum-alloy 12, Cupro-nickel, Everdur, Waukesha metal, and acid, aluminum, manganese, and phosphorous bronzes. In certain tests these metals were rather heavily corroded, but in most instances the losses were small. Cupro-nickel and Waukesha alloy tended to become passive by forming blue-gray protective films which were not observed on other metals. A third group of

metals and alloys which lost over 2.4[1] mg/dm²/d in at least one-third of the tests consisted of Aluminum-alloy 55, Aluminum 86, chrome plate, Monel metal, and silver. A fourth group consisting of metals and alloys losing over 2.4 mg/dm²/d in at least half of the tests included cast iron, machine brass, nickel, nickel plate, Niresist, steel, tin, and tin plate. This group was the least resistant of all metals and alloys tested, and for this reason only a limited number of tests was made with some of the metals.

All metals except the stainless steels, Durimetl, and Inconel discolored in various ways during the tests. There was no evidence of deep pitting although cast iron, steel, and Niresist always appeared slightly etched after exposure. Copper, tin, and Aluminum-alloys 12 and 55 and Aluminum 86 appeared slightly etched after exposure under one of the crushers. Tin and nickel samples exposed in filter chargers showed the erosive effects of moving particles.

Relation of Composition of Metal to Corrosion Resistance: There was no definite correlation between resistance and the composition of the metals tested although a few observations were made. All stainless steels tested, regardless of composition, were among the most resistant metals tested. Aluminum-alloy 76, containing two per cent of added nickel, was generally more resistant than the other aluminum alloys or pure aluminum. The copper-nickel combinations present in Cupro-nickel and Waukesha alloys were relatively resistant in most instances. Waukesha alloy, which was somewhat less resistant than Cupro-nickel alloy, contained some iron, whereas the Cupro-nickel alloy was free of iron. Monel metal contained less copper and more nickel than Cupro-nickel or Waukesha alloys and was less resistant than these two metals. Machine brass and manganese bronze, which contained zinc, were relatively non-resistant copper alloys.

Effect of Location in the Winery on Corrosion: The greatest amount of corrosion occurred at the two crushers (Table 4) where aeration and the rapidity with which the juice and must moved over the metals were corrosion factors. The exposure of metals, at the crushers, to unfermented juice may also have been an important factor, since Searle, *et al.* (1934) found that corrosion in fresh juices was more rapid than in fermented juices. The most resistant metals and alloys tested at the crushers were the stainless steels, Cupro-nickel, and Inconel. Some metals were corroded more severely at the red-grape crusher. Aluminum 86, Aluminum-alloy 55, Inconel, Monel metal, nickel plate, silver, and silver plate were more severely corroded when exposed at the red-grape crusher; whereas aluminum, acid,

[1] 2.4 mg/dm²/d was used because of its convenience for separating the metals into groups.

TABLE 3

Weight-Loss Distributions Showing General Resistance of Metals and Alloys to All Test Conditions[1]

Losses in mgs/dm²/d	Aluminum LB	Aluminum 86	Aluminum 55	Aluminum 12	Aluminum 76	13 Aluminum bronze	18 Acid bronze	19 Cupro-nickel	11 Everdur	16 Machine brass	15 Manganese bronze	14 Phosphorous bronze	10 Waukesha alloy	Nc Inconel	33 Niresist	M Monel metal	N Nickel
0 0.4	2	2	....	5	4	6	4	9	7	2	3	6	6	15	....	4	....
0.5- 0.9.....	3	1	2	2	2	3	3	3	1	2	2	2	3	....	....	4	1
1.0- 1.4.....	2	1	1	6	6	2	5	3	3	1	2	2	....	1	....	1	2
1.5- 1.9.....	4	....	1	1	3	1	1	....	2	1	2	2	3	....	....	....	1
2.0- 2.4.....	....	1	1	....	1	1	....	....	1	1	2	1	1	....	....	1	2
2.5- 4.9.....	5	1	2	....	....	3	2	....	1	4	4	2	2	....	1	2	1
5.0- 7.4.....	....	....	....	....	....	....	....	1	....	1	....	....	....	....	....	....	3
7.5- 9.9.....	....	....	....	....	....	....	1	....	....	....	....	....	1	....	....	2	1
10.0-19.9.....	....	1	....	1	....	....	....	....	1	2	1	1	....	....	1	1	1
20.0 29.9.....	....	1	1	....	....	....	....	....	....	....	....	....	....	....	....	....	1
30.0-39.9.....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1	1	....
40.0-49.9.....	....	....	....	....	....	....	....	....	....	1	....	....	....	....	1	....	1
Over 50.....	....	....	....	....	....	....	....	....	....	1	....	....	....	....	1	....	2

[1] The number of tests showing the losses listed in the first column.

TABLE 3 *(Concluded)*

Losses in mgs/dm²/d	C Copper	21 Tin	22 Silver	UL 18-8 Alloy	17 Cast iron	20 Steel	56 Nickel-plated copper	57 Tin-plated copper	58 Chromium-plated copper	59 Silver-plated copper	111 Allegheny C pickled	44 Allegheny C cold-rolled	N 2795 Durimet	Stainless steels S1—S13	110 Metallized aluminum	120 Metallized tin	130 metallized copper
0 0.4.....	2	4	1	15	....	....	1	....	5	4	8	8	8	16	8	8	8
0.5- 0.9.....	2	....	3	1	....	....	1	1	1	1	....	....	....	....	....	....	....
1.0- 1.4.....	4	....	....	....	....	....	2	....	....	2	....	....	....	....	....	....	....
1.5- 1.9.....	4	....	....	....	....	....	....	1	....	....	....	....	....	....	....	....	....
2.0- 2.4.....	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
2.5- 4.9.....	2	2	....	....	....	....	1	1	....	....	....	....	....	....	....	....	....
5.0- 7.4.....	....	1	1	....	....	....	....	....	2	....	....	....	....	....	....	....	....
7.5- 9.9.....	....	....	....	....	....	....	....	2	....	....	....	....	....	....	....	....	....
10.0-19.9.....	....	3	....	....	....	1	....	3	....	....	....	....	....	....	....	....	....
20.0-29.9.....	1	2	1	....	....	1	1	....	....	....	....	....	....	....	....	....	....
30.0-39.9.....	....	....	....	....	....	2	....	....	....	1	....	....	....	....	....	....	....
40.0-49.9.....	....	1	....	....	....	1	....	....	....	....	....	....	....	....	....	....	....
Over 50.....	....	3	....	....	8	3	2	....	....	....	....	....	....	....	....	....	....

manganese, and phosphorous bronzes, Aluminum-alloy 12, copper, Everdur, machine brass, and Waukesha alloy were more severely corroded at the white-grape crusher. Searle, *et al.* stated that tests conducted in open and in closed fermenters support the conclusion of Culpepper and Caldwell (1927) that the anthocyanin of red juice accelerates the corrosion of tin, but they did not confirm the suggestion of Mrak and Cruess (1933) that anthocyanin accelerates the corrosion of nickel and Monel metal. The data discussed above show that nickel, nickel plate, and Monel metal corroded more rapidly at the red crusher than at the white crusher (Table 2). Similar data for red and white fermenters, however, were not consistent with these. Tin corroded at about the same rate at both the red and white crushers but more rapidly in the red fermenter than in the white fermenter. When SO_2 was added to the fermentation tanks, tin corroded more rapidly in the white fermenter.

The stainless steels were resistant under all conditions tested in fermenting musts. The most resistant of other metals tested in fermenting white juice free of SO_2 were aluminum and acid and phosphorus bronzes. Aluminum and phosphorus bronzes, Cupro-nickel, and Waukesha alloy did not show perceptible losses when exposed in fermenting white juice containing SO_2. The most resistant metals, other than the stainless steels, tested in red juice free of SO_2 were aluminum, aluminum bronze, and Cupro-nickel. Phosphorous bronze was the only metal or alloy, other than the stainless steels, that did not show a perceptible loss when tested in fermenting red juice containing SO_2. Corrosion was greater in most tests in white must than in red must; although machine brass, tin, and steel were corroded more severely in the unsulfured red juice than in the unsulfured white juice. When sulfurous acid was added to the fermenting juices, the corrosion of machine brass, copper, and steel in white must was increased but was decreased in red must. Cupro-nickel and Waukesha alloy on the other hand corroded more when SO_2 was added to the white must. The corrosion of nickel and cast iron by both red and white must was increased on addition of SO_2. The addition of SO_2 to white must greatly increased the corrosion of tin, whereas the addition of SO_2 to red must tended to decrease it. Aluminum bronze was very resistant in all fermentation tests except when SO_2 was added to red must.

The corrosion of metals and alloys in sumps was not great although all metals except the stainless steels showed perceptible losses. The stainless steels and Inconel were the most resistant. Waukesha alloy was resistant in the white sump but showed a perceptible loss in the red sump.

Metals and alloys exposed in storage tanks were not heavily corroded, and the amount of corrosion varied with the type of storage. Most of the test pieces stored in Sauterne wine lost more weight than when exposed in other wines. Port wines were usually less corrosive than other stored wines. The stainless steels and Inconel were relatively resistant to all storage tests. Other relatively resistant metals were the following: in Sauterne, Aluminum-alloy 76; in new white wine, Aluminum 86; in Zinfandel red wine, Cupro-nickel, Monel metal, and silver plate; in sherry stock, silver; and in port, Cupro-nickel, Everdur, and Waukesha alloys.

The stainless steels, Waukesha alloy, and Inconel were the most resistant in the two sherry cooker tests, although corrosion was relatively light in most instances. Most metals corroded more in the sherry cooker at 43.33°C.(110°F.) than in the sherry cooker at 56.67°C.(135°F.). Searle, *et al.* explain a higher rate of corrosion on the basis of the higher concentration of oxygen at the lower temperature. In this case, however, the differences observed cannot safely be ascribed to temperatures alone since the tests were conducted in different cookers with different musts.

The corrosion of metals in the filter charger was usually considerable because of agitation, aeration, and erosion. The stainless steels and Inconel were the most resistant of the metals and alloys tested at this point.

Use of Metals in Wineries: In view of the data presented in this paper it is apparent that very few metals are resistant to corrosion under all conditions existing in a winery. In choosing a metal, therefore, the only safe criterion for selection is a test conducted in the winery at the place where the metal is to be used. Although the safest procedure is to use the most resistant metal indicated by such tests, it is not always the most feasible procedure. In many instances it is possible to use less resistant metals in the winery provided they are used in places where they come in contact with the juices and wines during the early stages of manufacture. Byrne, Saywell, and Cruess (1937) have shown that much of the iron content of musts is lost during fermentation. Furthermore, Benvegnin and Capt (1934) found that copper is deposited during fermentation and storage. According to these findings it appears that reasonably resistant metals are safe to use during crushing and fermentation, but the most resistant metals should be used in contact with stored and finished wines.

SUMMARY AND CONCLUSIONS

1. The corrosion of 46 metals and alloys at various stages of the process of making wines in California has been determined. Corrosion varied with the metal used and the conditions of the test.

TABLE 4

Weight-Loss Distribution of All Metals Showing Relative Severity of Corrosion at Various Points in the Winery[1]

Losses of metals corroded in mg/dm²/d	White crusher	Red crusher	White sump	Red sump	White fermenter	White fermenter +SO_2	Red fermenter	Red fermenter +SO_2
0- 0.4	4	2	6	6	12	8	10	8
0.5- 0.9	1	1	3	1	2	2	4	3
1.0- 1.4	....	5	3	2	2	2	4	2
1.5- 1.9	1	2	3	5	1	1	....	....
2.0- 2.4	....	1	2	....	....	....	....	....
2.5- 4.9	2	2	2	1	3	2	....	2
5.0- 7.4	2	1	1	1	....	....	....	1
7.5- 9.9	2	1	1	1	....	....	....	....
10.0-19.9	7	....	....	....	1	1	3	1
20.0-29.9	2	4	....	....	....	....	....	1
30.0-39.9	....	3	....	1	....	....	1	....
40.0-49.9	2	....	1	....	....	....	....	....
Over 50	3	4	1	1	1	3	1	1

[1] The number of tests showing the losses listed in the first column.

TABLE 4 *(Concluded)*

Losses of metals corroded in mg/dm$_2$/d	Port storage	Sherry stock	New dry red	New dry white	Sauterne storage	Filter charger	Sherry cooker 43.33°C. (110°F.)	Sherry cooker 56.67°C. (135°F.)
0- 0.4	10	5	6	5	4	3	8	12
0.5- 0.9	1	5	4	4	....	4	6	2
1.0- 1.4	3	8	6	3	1	2	2	2
1.5- 1.9	2	2	....	4	....	2	2	2
2.0- 2.4	2	....	1	2	1	3	....	1
2.5- 4.9	....	3	2	2	8	6	1	....
5.0- 7.4	....	....	....	1	1	....	1	....
7.5- 9.9	....	....	1	....	1	....	....	....
10.0-19.9	....	2	....	....	....	....	1	1
20.0-29.9	....	....	....	....	1	1	....	....
30.0-39.9	....	....	....	....	....	....	....	1
40.0-49.9	1	....	....	....	1	....	....	....
Over 50	....	....	1	....	....	3	....	....

2. The stainless steels, Inconel, and Aluminum-alloy 76 were the most resistant metals tested. Cast iron, steel, tin, Niresist, and nickel were the least resistant metals tested. The metallized metals and Durimetl were resistant, but this may have been due to tartrate deposits.

3. Corrosion was most severe at the crushers. Metals exposed under the white crusher were usually more heavily corroded than when exposed under the red crusher. The filter charger also exerted a strong corrosive action on most metals and alloys because of agitation, aeration, and erosion. The white fermenter was more corrosive than the red fermenter.

4. The addition of SO_2 during fermentation increased corrosion in some instances and decreased it in others.

5. White juices or wines were usually more corrosive than the red juices or wines. Monel metal, nickel, nickel plate, silver, and silver plate corroded more at the red crusher, but these differences were not consistent in all tests.

6. The behavior of a metal or alloy under a certain set of conditions can be definitely ascertained only by tests under those conditions; for example, out of a group of metals and alloys one might prove most resistant at the crusher and another in finished dry wine.

ACKNOWLEDGMENTS

The writers wish to thank the Concannon Vineyards, the Roma Wine Company, and Shewan Jones Ltd., for permitting tests to be made in their wineries. They also wish to express their appreciation of the coöperation and assistance received from the International Nickel Company, the Pacific Brass Foundry of San Francisco, the Columbia Steel Company, the Aluminum Company of America, Dr. W. V. Cruess, Dr. M. A. Joslyn, and Mr. G. L. Marsh.

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OAT FLOUR AND HEXANE EXTRACT OF OAT FLOUR AS ANTIOXIDANTS FOR SHELLED WALNUTS AND WALNUT OIL[1]

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(Received for publication, August 19, 1937)

The oxidative deterioration of fats and oils has been found to be similar to other autocatalytic oxidations in its general aspects, particularly in regard to the catalytic influence of traces of certain foreign substances, according to Moureau and Dufraise (1927), Milas (1932), and Barmore (1936). A variety of substances have been found to inhibit this oxidative deterioration, particularly by Greenbank and Holm (1934), but with few exceptions the most efficient antioxidants are of synthetic origin and of questionable safety when used in edible fats and oils. Musher (1935), however, found that cereals, particularly oat, have definite antioxidative powers; and the use of oat flour has been suggested for a variety of food products by Peters and Musher (1937) and Lowen, Anderson, and Harrison (1937).

Shelled nut meats have been marketed in increased quantity of late, and the usual methods of packaging in vacuum or under an inert gas atmosphere are not entirely satisfactory in preventing rancidification. Therefore, at the suggestion of Musher we investigated the possibility of preventing the rancidification of shelled walnuts by the use of oat flour (Avenex 7) and of hexane extract of oat flour (Avenol). In view of the difficulty of getting intimate mixing of oat flour with the oil in the walnut meats, it was decided to test the effect of the preparation on the oil itself in order to better study their antioxidative effects. In addition, the antioxidative power of Avenex and Avenol was compared to that of other antioxidants on the rancidification of olive and cottonseed oils.

MATERIALS USED

The shelled walnuts were obtained from the California Walnut Growers Association. The walnut oil was obtained from the Pacific Nut Company in Los Angeles and was freshly expressed.

The oat flour and hexane extract of oat flour (decorticated oat flour) were supplied by the Quaker Oats Company through the facili-

[1]This investigation was aided by a grant from the Musher Foundation, Inc.

ties of the Musher Foundation, Inc. These were Avenex and Avenol (hexane extracts).

EXPERIMENTAL PROCEDURE

In order to study the effectiveness of an antioxidant with reasonable dispatch, a certain amount of acceleration is required. This was obtained by storing the samples treated in an incubator at 40°C. (104°F.). The samples were also stored in the dark so as to avoid the effect of light [compare Coe (1934) and Coe and Le Clerc (1934)].

The progress of the oxidation was followed by determining the peroxide value. The Kreis test for rancidity was used also, and in later tests the changes in the iodine number were followed.

The technic for measuring peroxides was essentially that of Wheeler (1932). Two-gram samples were accurately weighed out and dissolved in 30 ml. of solvent mixture (60 per cent glacial acetic acid and 40 per cent chloroform by weight); .5 ml. of saturated KI solution was added and the mixture rotated gently for one minute, after which 100 ml. of distilled water was added and the liberated iodine titrated with 0.02N sodium thiosulfate using starch indicator. The results were expressed as mols of peroxide, being calculated as follows:

$$\text{Mols of peroxide} = \frac{.5\ (\text{ml. } Na_2S_2O_3 \text{ used} \times \text{normality of } Na_2S_2O_3)}{\text{Grams of oil}}$$

The Kreis test for rancidity of oils was carried out as given by Jamieson (1932). Ten ml. of oil was shaken with 10 ml. of concentrated HCl for 30 seconds in a large, rubber-stoppered test tube. Then 10 ml. of .1 per cent phloroglucinol in ether was added and the tube again well shaken. A red color developing on addition of the phloroglucinol was considered positive.

The Hanus method was used for determining iodine absorption number as given in the A.O.A.C. (1935), .25 gm. of oil being placed in a 500-c.c. glass-stoppered bottle and 10 ml. of $CHCl_3$ added. Then 25 ml. of the Hanus iodine solution was added and the mixture allowed to stand 30 minutes with occasional shaking. At the end of 30 minutes 10 ml. of 15 per cent KI solution was added and then 100 ml. of distilled water. The iodine was titrated with 0.1N $Na_2S_2O_3$ with starch indicator. Blank determinations were made with the sample. The iodine number was calculated as follows:

$$\text{Iodine number} = \frac{(\text{Blank titration} - \text{Sample titration}) \times \text{N of } Na_2S_2O_3 \times .0127 \times 100}{\text{Grams of oil}}$$

RESULTS AND DISCUSSION

Shelled Walnuts Treated With Avenex and Avenol: The shelled walnuts were prepared with Avenex in two ways. In one the walnut meats were used as received and in the other they were finely ground with a food chopper. Each lot was divided into two series, one being stored at room temperature and the other at 40°C.(104°F.). The shelled, ground samples were stored with .5 per cent and three per cent Avenex respectively, and the unground samples with one per cent and five per cent Avenex. The Avenex was applied by dusting it over the walnuts and thoroughly mixing them so as to get uniform distribution.

In the Avenol treatment only shelled, unground walnuts were used. Two series were stored as in the case of the Avenex-treated walnuts. They were treated with .2 per cent Avenol and .4 per cent Avenol. As the amount of Avenol used was small in comparison with the sample of walnuts treated, it was found unsatisfactory to apply Avenol directly since it was impossible to get it evenly distributed over the walnuts. Therefore, the Avenol was taken up in petroleum ether and by means of a De Vilbiss spray was sprayed over the meats, care being taken to mix the meats and the solution thoroughly. The ether was removed by placing the treated samples in a current of warm air until no trace of ether odor could be detected.

The walnut samples were all stored in partly filled, two-quart jars which were loosely sealed to permit free access of air.

To determine the peroxide value it was first necessary to remove the oil. After trying several methods a cold extraction of a ground sample of walnuts with petroleum ether was found to give the most satisfactory results. The method in brief was to place a 25-gram, finely ground sample in a cork-stoppered bottle with 100 ml. of petroleum ether. The sample was allowed to stand 24 hours and the supernatant liquid decanted off. The sample was then washed with 25 ml. of petroleum ether on three consequent times at two-hour intervals. The petroleum ether was removed from the extracts under vacuum. The peroxide value was then determined and the results reported as mols of peroxide x 10^3 per 25-gram sample (Table 1).

The first indication of rancidity in the walnuts was noticed organoleptically after 35 days in Series 4 and 5, the unground walnut samples, but was not pronounced until after 56 days. Samples 2 and 2a became slightly rancid in 110 days but no trace of rancidity was observed in any of the other samples at this time. The peroxide values were not determined then.

Walnut Oil Treated With Avenex and Avenol: The first series of tests on walnut oil treated with Avenex and Avenol was conducted

TABLE 1

Peroxide Value of Oil in Treated Shelled Walnuts

Sample No.	Treatment	Storage temperature	Mols peroxide x 10^3 per 25 grams											
			Days stored											
			0	5	8	12	15	19	26	35	40	56	70	86
		°C.												
1	Ground, no Avenex	Room	.....	6.5	4.9	5.5	5.1	6.8	4.7	4.1	7.4	2.2	6.7	7.9
2	Ground, no Avenex	40	23.2	6.0	5.0	6.0	4.4	4.6	5.7	6.0	5.1	4.0	11.4	11.6
1a	Ground, ½% Avenex	Room	25.5	4.8	5.6	5.2	3.1	3.6	3.5	3.2	4.4	2.1	5.2	7.3
2a	Ground, ½% Avenex	40	25.8	7.4	5.2	4.7	4.6	5.0	5.7	3.9	6.4	4.3	8.1	10.0
1b	Ground, 3% Avenex	Room	23.8	4.5	2.9	3.9	1.8	2.5	2.4	2.3	2.1	1.8	4.7	6.5
2b	Ground, 3% Avenex	40	23.5	6.2	4.7	6.7	4.3	6.2	7.7	5.0	4.9	3.6	8.4	9.3
3	Unground, no Avenex	Room	26.1	6.0	5.7	5.0	4.2	5.0	5.8	5.5	3.9	4.7	.....	5.7
4	Unground, no Avenex	40	18.1	6.2	4.6	8.9	3.5	6.4	7.2	12.3[1]	7.5[1]	11.2[3]	.....	25.6[3]
3a	Unground, 1% Avenex	Room	26.6	5.4	4.5	6.9	4.1	4.7	5.2	5.0	3.4	3.8	.....	6.0
4a	Unground, 1% Avenex	40	20.4	6.0	4.6	7.1	3.4	4.2	5.8	19.3[1]	8.3[1]	7.3[2]	.....	18.2[3]
3b	Unground, 5% Avenex	Room	24.4	6.2	4.0	7.2	4.1	4.9	4.6	4.8	5.5	4.1	.....	6.3
4b	Unground, 5% Avenex	40	21.5	5.1	4.8	5.6	3.3	8.1	6.7	17.0[1]	7.1[1]	7.4[2]	.....	15.4[3]
5	Unground, .2% Avenol	40	20.7	5.1	5.3	6.4	3.4	7.3	5.8	15.1[1]	10.78[1]	10.72[1]	.....	21.0[3]
5a	Unground, .4% Avenol	40	26.3	5.1	5.4	.....	3.5	8.0	7.8	12.4[1]	6.7[1]	8.2[1]	.....	18.4[3]
6	Unground, .2% Avenol	Room	26.9	6.2	4.9	6.5	3.5	8.6	4.7	11.7	3.2	2.8	.....	13.9
6a	Unground, .4% Avenol	Room	19.3	5.1	4.2	5.3	3.3	7.6	3.7	7.4	2.5	2.9	.....	7.9

[1] Slight rancidity. [2] Rancid. [3] Very rancid.

as follows: Forty grams of oil were placed in 50-ml. Erlenmeyer flasks. In the Avenex-treated samples four series were prepared; two were tightly stoppered with cork stoppers and two were plugged with cotton. One series of each was stored at room temperature and one at 40°C. (104°F.) One sample in each case was held as a control and the others treated with one and five per cent Avenex respectively.

In the Avenol-treated samples two series were prepard. They were both tightly stoppered, one series being stored at room temperature and one at 40°C. The samples were treated with one-half and one per cent Avenol respectively.

The Avenex-treated samples stored at room temperature showed a complete retardation of peroxide formation, while the untreated samples showed an increase, although they were not rancid (Tables 2 and 3). In the samples stored at 40°C. peroxide formation was slightly retarded when one per cent of Avenex was added and almost completely retarded when five per cent was added. The control samples were quite rancid.

In the Avenol-treated samples no rancidity was noticed organoleptically in the samples stored at room temperature; however, the Avenol retarded peroxide formation. In the samples stored at 40°C. the peroxide formation was not greatly retarded and all were rancid at 60 days. Sample 6a became very rancid at 74 days and the peroxide value increased markedly over the others. No explanation can be offered for the sudden rise of the peroxide value as this was not observed in a later series.

Comparison of Avenex and Avenol With Other Antioxidants in Inhibiting Rancidification of Walnut Oil, Olive Oil, and Cottonseed Oil: A large number of antioxidants have been suggested for use with oils. Moureau and Dufraise (1927) have stressed particularly the value of hydroquinone, alphanaphthol is commonly used in industrial oils, and maleic acid has been recommended by Greenbank (1933) and Greenbank and Holm (1934). Of the nitrogenous derivatives the acyl derivatives of weakly basic nitrogen compounds have been recommended for oils by Mardles and Helmore (1935), but the ethanolamines, whose use has been suggested by Murrill (1931), were found to be extremely efficient for olive oil by Ball (1936). The latter also reported that triethylamine was a good antioxidant although of objectionable fishy odor; therefore in our comparison of antioxidants these substances were tested.

The technique used in this comparison was the same as that for the walnut-oil samples mentioned previously. The oils were treated with Avenex, Avenol, hydroquinone, alphanaphthol, maleic acid, and triethylamine (Tables 4, 5, and 6).

TABLE 2

Peroxide Value of Walnut Oil Treated With Avenex

Sample No.	Stopper	Treatment	Storage temperature	Mols of peroxide x 10^3/gm. oil														
				Time in days														
				0	6	13	17	19	24	27	32	34	36	39	41	43	47	56
			°C.															
1.....	Cork	No Avenex	Room	11.90	9.04	11.30	12.90	11.60	8.22	23.20	20.80	21.20	28.60	19.20	14.70	24.10	20.05	22.51
2.....	Cotton	No Avenex	Room	12.40	8.35	12.90	13.00	10.50	8.65	18.00	19.30	20.10	26.00	16.90	13.00	20.70	18.25	19.41
1a.....	Cork	1% Avenex	Room	9.48	4.99	8.51	9.02	6.02	2.33	12.80	10.10	8.90	20.50	9.30	8.00	15.04	9.31	7.61
2a.....	Cotton	1% Avenex	Room	8.83	5.24	8.29	9.95	5.89	2.32	12.40	10.80	10.30	21.50	9.20	7.90	15.50	9.29	7.60
1b.....	Cork	5% Avenex	Room	5.48	1.24	8.25	6.19	2.50	0.32	9.80	3.40	3.10	14.90	6.10	4.30	8.23	5.12	3.75
2b.....	Cotton	5% Avenex	Room	5.48	2.04	9.71	7.82	1.52	0.46	11.50	4.60	5.50	.....	4.00	4.20	7.79	4.96	4.82
3.....	Cork	No Avenex	40	11.20	8.23	14.30	16.00	14.10	15.70	31.70	34.10	35.90	44.60	40.60	29.40	39.20	40.42	56.00
4.....	Cotton	No Avenex	40	9.95	9.33	21.00	23.10	18.90	20.10	31.90	46.20	46.70	54.90	53.70	37.40	48.00	51.80	82.18
3a.....	Cork	1% Avenex	40	9.94	5.06	12.50	14.50	7.92	3.89	18.80	14.50	17.10	27.20	19.10	14.50	24.20	22.08	30.88
4a.....	Cotton	1% Avenex	40	8.46	4.91	15.70	14.30	7.16	4.26	19.20	16.70	17.30	23.80	17.20	14.70	24.90	22.38	32.00
3b.....	Cork	5% Avenex	40	4.69	1.85	8.79	9.59	1.78	0.56	10.80	5.80	6.50	12.70	3.50	4.00	10.20	4.92	6.32
4b.....	Cotton	5% Avenex	40	3.90	1.76	6.75	14.40	1.41	0.56	8.80	7.10	4.90	10.00	3.20	4.10	10.88	4.82	5.40

TABLE 3

Peroxide Value of Walnut Oil Treated With Avenol

Sample No.	Treatment	Storage temperature	Mols of peroxide x 10^2/gm. oil													
			Days stored													
			0	2	4	8	15	17	22	24	28	30	39	44	53	60	74
		°C.															
5	None	Room	2.6	4.6	7.8	7.8	7.8	10.6	19.4	10.8	10.0	9.4	12.6	21.6	13.5	15.5	19.3
5a	.5% Avenol	Room	1.0	2.3	2.7	2.3	2.9	4.0	13.0	5.0	3.0	1.8	4.5	14.9	4.0	6.5	9.2
5b	1% Avenol	Room	0.7	1.8	1.9	1.7	3.1	2.7	7.9	4.5	1.7	0.8	3.2	9.9	2.0	3.8	4.7
6	None	40	2.0	5.5	7.1	8.0	9.8	12.6	25.5	15.7	15.8	16.8	28.6	39.6	43.4	55.1[1]	81.8[1]
6a	.5% Avenol	40	0.9	3.0	4.4	6.1	7.7	12.6	23.1	13.6	15.6	16.2	30.8	44.6	45.6	59.2[1]	144.5[2]
6b	1% Avenol	40	0.7	2.0	2.2	2.6	5.7	5.8	13.0	7.5	8.0	7.4	15.8	29.8	25.7	36.8[1]	78.8[1]

[1] Rancid organoleptically. [2] Very rancid organoleptically.

TABLE 4

Comparison of Other Antioxidants With Avenex and Avenol in Walnut Oil

Sample No.	Treatment	Storage temperature	Mols peroxide x 10^2/gm. oil											Kreis test[2] 1 + 9 dilution
			Days stored											
			0	6	13	20	29	36	42	49	56	63	77	
		°C.												
a	None	Room	5.9	4.0	7.0	10.8	8.7	10.4	11.4	12.3	14.31	15.4	17.2	+
a1	None	40	5.2	3.4	8.1	12.6	15.1	20.4	23.8	26.0	31.0	34.7	36.1	+++
b	1% Avenex	40	3.2	1.8	3.7	8.2	8.6	14.4	19.3	21.0	26.9	32.4	32.9	+±
b1	5% Avenex	40	2.5	0.9	1.7	3.6	3.0	3.7	5.7	4.4	3.5	6.5	5.1	—
c	.5% Avenol	40	3.8	1.5	3.9	10.0	13.1	20.0	25.9	31.0	36.0	39.4	38.0	++
c1	1% Avenol	40	3.0	3.2	8.1	16.0	17.6	20.4	25.0	25.0	29.1	33.3	34.2	++
d	.01% Hydroquinone	40	5.8	3.7	6.6	10.6	10.3	12.7	14.3	14.0	16.1	18.3	18.9	+
d1	.1% Hydroquinone	40	6.7	2.8	5.1	9.5	8.5	10.3	10.9	10.6	12.5	14.3	13.3	+
e	.01% α Naphthol	40	7.3	4.0	6.1	12.6	14.8	19.1	22.2	24.0	24.3	33.7	35.6	+±
e1	.1% α Naphthol	40	5.9	3.3	7.0	13.1	10.9	14.4	17.2	17.9	21.5	25.1	28.5	+±
f	.01% Maleic acid	40	7.8	3.9	8.1	12.5	14.1	19.7	25.7	29.9	37.3	45.6	78.8[1]	++++
f1	.1% Maleic acid	40	5.8	3.6	6.7	12.9	10.2	13.3	15.7	16.5	19.5	22.9	25.1	+++
g	.01% Triethylamine	40	6.8	2.7	5.4	9.2	8.5	12.0	15.2	15.6	19.0	26.5	29.1	+±
g1	.1% Triethylamine	40	4.5	1.2	3.0	6.1	3.5	4.8	6.5	5.5	6.0	9.3	12.2	—

[1] Rancid organoleptically. [2] — No color; + light pink color; ++ medium pink color; +++ dark pink color; ++++ red.

TABLE 5

Comparison of Other Antioxidants With Avenex and Avenol in Olive Oil

Sample No.	Treatment	Storage temperature	Mols peroxide x 10^3/gm. oil										Kreis test 1 + 9 dilution
			Days stored										
			0	7	14	21	29	37	44	51	59	80	
		°C.											
a	None	Room	7.8	12.4	9.1	9.9	9.9	13.1	13.6	13.6	15.0	15.3	+
a1	None	40	8.4	13.5	11.9	13.3	14.5	17.8	20.0	20.5	23.8	31.0	++
b	1% Avenex	40	3.3	7.1	.....	3.0	3.9	6.0	6.8	6.8	8.6	9.5	—
b1	5% Avenex	40	0.4	1.3	0.8	0.5	0.5	1.4	1.6	0.6	1.6	0.7	—
c	.5% Avenol	40	2.0	4.7	1.8	1.9	2.7	4.2	5.5	5.8	7.0	7.8	—
c1	1% Avenol	40	0.8	1.1	0.6	0.6	0.6	1.4	1.5	0.8	2.0	2.2	—
d	.01% Hydroquinone	40	7.9	11.2	8.4	9.2	9.8	11.8	12.6	12.2	12.6	13.4	+
d1	.10% Hydroquinone	40	7.2	10.9	7.9	8.4	8.7	10.0	10.6	9.9	11.0	10.7	+
e	.01% α Naphthol	40	8.1	14.6	10.6	11.3	12.1	14.4	16.5	15.5	17.1	17.8	++
e1	.10% α Naphthol	40	7.7	11.5	8.9	9.8	10.6	12.4	13.2	12.8	14.6	14.6	+
f	.01% Maleic acid	40	8.0	13.8	10.4	11.3	12.0	14.3	15.7	14.8	16.8	19.6	++
f1	.01% Maleic acid	40	7.6	12.0	9.4	8.8	8.9	10.2	10.6	9.6	10.1	9.0	+
g	.01% Triethylamine	40	8.0	10.5	6.4	6.2	5.7	7.1	7.9	7.0	8.1	7.5	—
g1	.10% Triethylamine	40	7.1	5.5	2.3	1.6	0.9	1.1	0.9	0.7	1.0	0.3	—

TABLE 6

Comparison of Other Antioxidants With Avenex and Avenol in Cottonseed Oil

Sample No.	Treatment	Storage temperature	Mols peroxide x 10^3/gm. oil										Kreis test 1 + 9 dilution
			Time in days										
			0	7	14	22	29	36	43	50	58	78	
		°C.											
a	None	Room	5.9	5.8	7.1	10.7	15.2	18.7	21.7	26.8	30.9	42.4	++
a1	None	40	6.0	8.2	10.3	16.3	23.8	33.2	37.3	51.1	61.3	69.5	+++
b	1% Avenex	40	5.4	1.9	3.1	5.0	9.3	14.1	18.1	23.6	31.2	37.7	++±
b1	5% Avenex	40	2.6	0.8	0.5	0.6	1.8	2.9	5.4	7.2	15.1	22.5	+
c	.5% Avenol	40	2.5	1.2	3.2	6.7	12.1	16.1	19.2	24.9	38.4	49.7	++±
c1	1% Avenol	40	1.0	0.8	0.6	2.4	7.7	12.7	16.9	22.0	33.1	39.8	++±
d	.01% Hydroquinone	40	3.7	4.9	6.8	10.1	14.9	18.4	21.6	26.0	31.1	40.6	++
d1	.10% Hydroquinone	40	3.4	7.8	4.7	6.5	8.3	10.1	11.3	12.8	15.1	18.9	±
e	.01% α Naphthol	40	6.3	8.0	10.0	14.7	20.8	27.6	21.7	40.0	49.9	53.1	++
e1	.10% α Naphthol	40	4.4	7.4	9.9	16.5	23.7	31.2	37.1	43.1	50.2	57.8	++
f	.10% Maleic acid	40	5.5	7.6	15.6	21.8	33.3	41.2	49.2	58.4	64.2	72.5	+++
f1	.10% Maleic acid	40	4.9	5.7	7.4	10.1	15.3	18.7	22.5	29.5	29.6	32.2	++±
g	.01% Triethylamine	40	8.2	6.4	7.6	9.2	10.4	10.3	9.6	8.9	8.6	6.3	—
g1	.10% Triethylamine	40	9.0	5.1	4.2	3.2	3.0	2.7	2.2	1.2	1.3	0.6	—

In the walnut-oil samples only at the high concentrations of Avenex and triethylamine was the Kreis test negative. The only sample that was rancid organoleptically was that treated with .01 per cent maleic acid.

In olive oil the peroxide formation was very definitely retarded by Avenex, Avenol, and triethylamine. None of the samples were rancid organoleptically.

In cottonseed oil only triethylamine retarded peroxide formation. The other samples had a bad odor and taste.

Additional Tests With Walnut Oil: In the foregoing data on the peroxide value of the treated and untreated oils, several peculiar and unexplainable trends in peroxide values were noted. In the nut-meat series the initial values of peroxide were abnormally high but dropped to a low value after storage for five days. Thereafter they jumped about. These abnormalities were not due to experimental error, for the results obtained at a given time could be duplicated readily. Furthermore, samples that were definitely rancid had a high peroxide value. The same peculiar jumping values were observed in walnut oil, to some extent in olive oil, but not in cottonseed oil. In view of the fact that samples which were rancid organoleptically did show a high peroxide value, the contention of Coe (1934) and Coe and Le Clerc (1934) that the peroxide value is not an index to the course of oxidation when conducted in the dark does not necessarily apply here. The continued rise in peroxide value, in spite of the fluctuations, is in accord with the results reported in the literature by Lowen, Anderson, and Harrison (1937) and Coe and Le Clerc (1934). It is of interest that the steady state concentration of peroxide, such as was definitely found by Hamilton and Olcott (1937) for methyl oleate in 52 hours, was not reached even after 110 days in our case. Furthermore, the relatively great resistance of the walnut oil to rancidification in spite of its higher unsaturation as compared with cottonseed and olive oil was interesting.

To obtain further data on these points the oxidation of walnut oil, alone and in presence of Avenex, was studied in more detail.

Two lots of these samples were prepared, 200 grams of walnut oil being placed in 300-ml. flasks and tightly stoppered. The first lot was used as a control; the second lot was treated with five per cent of Avenex. The samples were all stored at 40°C.

It is evident from the data (Table 7) that sampling errors might be involved in causing fluctuations in the results previously reported. This is shown by the variation in peroxide value between the three members of one lot. There was more fluctuation in peroxide value than in iodine number; however, the Avenex added did noticeably

TABLE 7

Rate of Oxidation in Walnut Oil

Sample No.	Treatment	Storage temperature	Iodine number									
			Time in days									
			0	7	14	21	28	35	42	56	67	124
		°C.										
1	None	40	150.5	151.4	150.6	145.6	151.5	152.0	150.5	150.0	149.5	148.0
	None	40	150.5	150.0	147.6	140.6	151.1	.....	150.5	150.0	149.5	148.0
2	None	40	150.5	149.4	.....	150.2	151.3	152.0	149.5	150.5	149.5	147.5
	None	40	152.0	150.5	149.5	.....	151.5	152.5	150.0	150.0	150.0	148.0
3	None	40	150.0	150.6	149.2	144.2	151.0	152.0	150.5	150.5	149.5	149.0
	None	40	.....	150.8	150.0	149.5	151.0	151.0	150.5	151.0	150.0	.....
4	5% Avenex	40	146.5	148.3	150.2	150.5	150.5	149.5	151.5	148.0	148.5	145.5
	5% Avenex	40	151.8	145.9	150.2	150.6	150.2	150.0	151.5	148.0	149.0	145.5
5	5% Avenex	40	150.5	150.6	150.4	150.5	150.7	150.5	149.0	149.0	146.0	146.5
	5% Avenex	40	151.0	150.4	150.6	151.0	150.3	150.5	149.5	147.5	148.0	146.0
6	5% Avenex	40	150.8	150.0	149.9	150.5	150.0	150.0	.....	150.0	149.0	.....
	5% Avenex	40	151.5	150.4	153.4	150.5	149.0	151.0	149.0	149.5	148.5	146.0

TABLE 7 *(Concluded)*

Sample No.	Treatment	Storage temperature	Mols peroxide x 10^3/gm. oil — Time in days										Kreis test	
			0	7	14	21	28	35	42	56	67	124	(1+19)	(1 + 9)
		°C.												
1	None	40	3.8	3.1	3.6	4.7	5.2	8.6	10.2	17.0	19.8	52.4	++	+++
	None	40	3.9	3.2	3.5	4.5	5.1	7.2	9.8	16.4	19.3	52.7		
2	None	40	3.8	3.1	3.3	4.2	5.2	8.1	8.8	15.8	19.1	47.3	+±	+++
	None	40	3.7	3.0	3.2	4.3	5.2	6.8	8.8	16.0	19.3	46.9		
3	None	40	3.7	3.3	3.3	4.5	5.6	7.4	9.6	12.2	19.0	18.1	—	+
	None	40	3.8	2.9	3.2	4.5	5.4	7.4	9.5	12.0	18.8	16.8		
4	5% Avenex	40	2.0	0.9	0.6	1.6	1.0	1.6	2.6	1.0	1.1	1.6	—	±
	5% Avenex	40	2.3	0.9	0.5	1.6	1.0	1.4	1.9	1.1	1.0	1.7		
5	5% Avenex	40	2.0	0.8	0.6	1.1	1.0	1.5	2.0	1.1	1.2	11.4	—	+
	5% Avenex	40	1.8	0.9	0.5	1.6	0.6	1.4	2.1	1.0	1.2	13.4		
6	5% Avenex	40	1.8	0.7	0.6	1.2	0.5	1.3	2.0	1.0	1.1	2.3	—	±
	5% Avenex	40	1.6	0.7	0.5	0.9	0.5	1.5	1.9	1.0	1.0	2.1		

inhibit the oxidation of walnut oil. The peroxide values were markedly reduced. The iodine number was slightly but definitely reduced in both the treated and untreated oils, more so in the treated. If this is to be construed as a greater reduction in the number of double bonds in presence of an antioxidant, the result is quite striking. This point is being investigated further.

SUMMARY

Oat flour and oat-flour extract were found to appreciably reduce the oxidation of walnut meats and oils. The flour was more active than the hexane extract. In order of decreased protection of walnut oil against oxidation the antioxidants used were as follows: .1 per cent triethylamine, 5 per cent Avenex, .1 per cent hydroquinone, .1 per cent maleic acid, and .1 per cent alphanaphthol. They behaved somewhat similarly towards cottonseed and walnut oil. Fluctuations and jumps were noted in peroxide values which may have been due to choice of original sample and sampling. The iodine number of walnut oil decreased but slightly with increase in peroxide value, this decrease being somewhat more in presence of Avenex.

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ERRATA: Generic names of fungi on pages 229, 232, and 233 in Vol. 2, No. 3 of Food Research should read *Streptococcus* instead of *Saccharomyces*.

CHARACTERISTICS OF AN ORGANISM CAUSING SPOILAGE IN FORTIFIED SWEET WINES

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(Received for publication, August 27, 1937)

Although reports exist in the literature concerning the bacterial contamination of dry wine containing from 12 to 14 per cent alcohol, very few reports are to be found describing similar types of spoilage in fortified sweet wines; i.e., wines to which brandy has been added, after the usual fermentation, to increase the alcohol concentration to 19 or 20 per cent. Such wines usually have been considered immune to bacterial spoilage because of their high alcohol content.

In the 1932 and later vintages of California fortified sweet wines frequent complaints have been received by this laboratory of a spoilage which is characterized by an extensive, flocculent, amorphous sediment in the bottom of the container with the supernatant liquid remaining perfectly clear. At no time does the wine, unless shaken severely, become turbid as it does with other bacterial diseases. Chemical examination of such wines reveals ordinarily that there has been but little change in composition. Upon prolonged storage at room temperature there is an increase in the volatile and fixed acids of the wine, together with a decrease in reducing sugars and the pH. An occasional sample is found in which some gas has been produced. Microscopic examination of the sediment from such wines reveals masses of long, intertwined filaments. Most frequently affected in the samples examined were muscatel, Angelica, and sherry types of wine, with occasional samples of port, Tokay, and Malaga. We have encountered no report of spoilage of this kind in dry wines.

The most frequent complaints in this type of disease are from instances in which spoilage occurs after final bottling. Although not affecting the taste greatly, diseased wine is unsightly and the consumer's reaction is naturally unfavorable. Such a reaction may be expected to affect future sales of similar or other types of wines from the same wineries. Since prolonged incubation is often necessary before spoilage takes place, the possibility exists that wine which is apparently sound upon leaving the winery may later develop the growth. The case histories of some of the complaints indicate that this condition prevails in many instances.

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Although the majority of reports of this type of spoilage have been upon bottled wines, positive cultures have been obtained from storage vats, shipping barrels, and tank cars, indicating that wine is often contaminated in the winery before bottling. The possibility exists, however, that at times sound wine may become diseased through the use of contaminated bottling equipment or other equipment with which the wine may come in contact.

EXPERIMENTAL PROCEDURE

Attempts to culture the causal organism, using the ordinary laboratory media, met with complete failure. In addition to nutrient broths, peptone solutions, brain, beef-heart, liver, and other standard media, various modifications of yeast-extract, grape-must, or tomato-juice infusions were tried without success (with and without change in pH, addition of sugar, alcohol, etc.). Growth has resulted only from the use of sweet wine diluted with an equal amount of water and sterilized by pasteurization, or by the use of one-per cent peptone or tryptone nutrient solutions to which at least 10 per cent by volume of sweet wine has been added. Growth in the same nutrient solutions, containing less than 10 per cent by volume of sweet wine, has been meager and inconsistent. The organism is cultivated with ease in the diluted wine medium, the growth becoming noticeable in the more rapidly growing strains after six days of incubation at room temperature. With some strains, however, growth does not occur until after a month or more of incubation. Comparative tests made by inoculating diluted wine media containing added nutrients, yeast infusion, tryptone, filtered tomato juice, or dried-liver particles, indicate that these substances do not improve the original medium.

By the addition of two per cent of agar to the diluted wine medium, colonies have been obtained with certain strains, and this method has been used in the purification of cultures. Growth in solid media is very poor, with only a very small percentage of inoculated tubes producing colonies. Growth could be obtained in solid media only by using deep agar shakes. Aerobic plates and anaerobic plate culture by the Spray (1930-31) technique or vegetable tissue jar, used by McClung, McCoy, and Fred (1934-35), with wine agar have not been successful.

Growth in dilution tubes of agar show that at least partially anaerobic conditions are necessary for the growth of this organism. Colonies rarely occur within less than one centimeter from the surface of the agar, with the majority of the colonies occurring in the center depths of the tube. In liquid cultures, growth occurs only at the bottom of the tube, with the supernatant liquid remaining clear.

The sediment produced varies from a coarse, granular sediment to an albumin-like, flocculent deposit. The formation of small, colony-like aggregates is frequently observed in young cultures. Upon vigorous shaking the deposit produced cannot be entirely dispersed but becomes suspended throughout the medium, producing a slight turbidity which clears upon standing a few hours.

Preliminary work on the nature of the acids produced in wine and the diluted wine medium indicates that lactic and acetic acids are produced, although acid production proceeds slowly. After 30 days' incubation at room temperature, only two or three cubic centimeters of N/10 acid are produced in 10 cubic centimeters of culture fluid. Gas production has not been observed in the artificially inoculated samples in contrast to the condition of certain original samples.

Using the previously mentioned diluted sweet-wine medium, the optimum pH and temperature relations were established. The optimum pH range lies between 4.1 and 4.3, with growth occurring from pH 3.7 to between 5.0 and 6.0. No growth has been obtained in the pH range of 6.0 to 7.0. A temperature of 20 to 25°C.(68 to 77°F.) is optimum, while temperatures above this range prolong the incubation period and in some cases inhibit growth altogether. After six days of incubation, for example, 24 of 32 strains tested were positive for growth at room temperature, 18 strains were positive at 30°C. (86°F.), and at 37°C.(98.6°F.) only 11 strains had grown. After 21 days of incubation all of the strains at room temperature had grown, while at 30 and 37°C., 28 and 25 strains, respectively, showed growth.

Microscopic examination of pure cultures reveals long, slender, intertwined filaments which upon being stained appear more or less segmented. A single filament may contain both gram-positive and negative segments. The microscopic appearance of fresh, unstained preparations resembles very closely a tangled mass of wet hair, and because of this characteristic the organism has earned the sobriquet of "hair bacillus" in the laboratory. True branching does not occur, and no spores or capsules have been demonstrated (Fig. 1).

DISCUSSION OF RESULTS

Investigation of control measures as reported by Douglas and Cruess (1936) shows that the addition of sulphur dioxide or citric acid will prevent this spoilage. In their experiments 60 parts per million of sulphur dioxide, or a total acidity of .5 gram per 100 c.c., was sufficient to prevent growth in sweet wine which had been inoculated with the organisms. Although no definite data have been obtained on the thermal death time of the organism, preliminary tests

indicate that it may be controlled by ordinary pasteurization temperatures. In addition to the results of the tests performed in the laboratory all of the available data that can be obtained from the industry indicate only a low heat resistance. The alcohol tolerance was found to be approximately 22 per cent by volume by inoculating diluted sweet wine containing added alcohol in varying concentrations. Spoiled samples of wine received at the laboratory have shown alcohol concentrations from 18.5 to 21.5 per cent by volume.

Apparently the only correlation between the chemical composition of the wine and its susceptibility to this type of spoilage is total acid

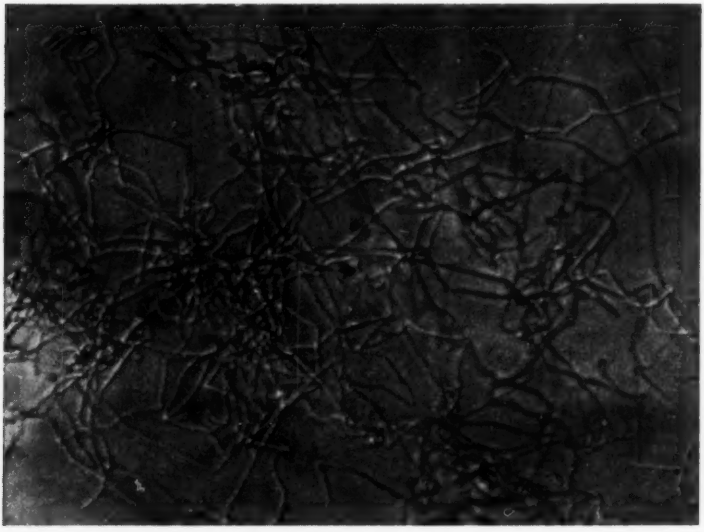

FIG. 1. Typical unstained microscopic preparation of organism causing flocculent amorphous sediment in sweet wines. (Magnification approximately 1,000.)

content; i.e., the lower the total acid, the greater its susceptibility. The percentage of sugar is not important, for the organism has been found growing in wines containing from .2 to 10 grams of reducing sugars per 100 c.c.

Referring to the literature on wine diseases, only a few reports have been found relating to bacterial spoilage of sweet fortified wines. Fevrier (1926) reported a bacterial disease in South African wines containing 18 per cent alcohol, and Niehaus (1932) reported "mannitic" bacteria in wines from the same locality. The development of an organism in Spanish port wine of 18 per cent alcohol was described by d'Estivaux (1935). Otani (1936) described four new species of

Lactobacilli capable of growing in sake (a Japanese beer) containing from 22 to 26 per cent alcohol. Fornachon (1936) isolated rod bacteria from fortified Australian wines and placed the organisms in the genus *Lactobacillus*.

With the exception of the last two reports, these papers have given but meager descriptions of the organisms involved, and none of those described characterize the type reported here. Certain vintners and wine chemists in California believe this spoilage is a variety of so-called "tourne" disease, or have suggested that in reports prior to 1932 this classification was given. We disagree with this viewpoint, however, believing that the peculiar cultural and morphological characteristics of this organism differentiate it from those isolated without difficulty in the "tourne" disease. The extreme difficulty with which consistent growth of this organism is obtained should perhaps be re-emphasized. This irregularity of growth has been a factor in the failure to indicate positively the origin of the organisms in contaminated wineries. Some attention has been given to this problem, but the results to date are not sufficiently conclusive to warrant an exact statement.

No attempts can be made at this time to indicate the exact systematic position of this organism as too little is known of the growth requirements and physiology. No report of an organism with such unusual morphology, together with such distinctive cultural characteristics, low optimum pH range, alcohol tolerance, sensitivity to temperatures much above room temperature, and inability to grow in the usual bacteriological media, has been found in this literature. The research on this organism is being continued in the laboratory.

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PRESERVATION AND STORAGE OF HENS' EGGS[1]

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(Received for publication, August 31, 1937)

About 53 per cent of the annual egg production in the United States occurs in the months of March, April, May, and June. Part of the eggs produced in the spring must be stored to stabilize the industry. About one-fifth of the eggs which enter the large markets during the year are stored in the shell or their contents are dried or frozen.

Each year about 270 million dozen eggs are stored in the shell and 90 million dozen are frozen. This corresponds to 33,000 carloads, having a market value of from 65 to 100 million dollars. Approximately four million pounds of dried yolk and two million pounds of dried white were imported in 1935. Egg drying in the United States fluctuates depending on the relation between the price of eggs in this country and in China, the rate of exchange, and the tariff. The uses for stored eggs in the various forms have been outlined in great detail (1).[2]

FROZEN AND DRIED EGGS

Whites, yolks, and mixed whole eggs are frozen (2). The white, which is used in candies, meringues, and cakes, is not altered appreciably by freezing. The physical properties of whole eggs which are used by bakers may be altered by freezing (3). Yolks are used in mayonnaise and noodle manufacture. Freezing precipitates or gels the lecitho-protein (4), the substance responsible for the emulsifying properties of the yolk (5). The freezing point can be lowered, and consequently the yolks can be stored at a much lower temperature if substances such as glycerine, sugars, or salt are added (4). If the yolk material with these substances added is actually frozen, precipitation occurs (6).

Yolks, whites, and mixed whole eggs are also dried (7). Drying may be done by tray or belt tunnel-dryers or by spray dryers. Spray-dried yolk is favored for some purposes, such as for ice cream (8). The large amount of entrainment loss in the outgoing air is a handicap in the spray drying of egg white. In order to obtain a dried

[1] Presented before the Division of Agricultural and Food Chemistry of the American Chemical Society September 10, 1936, at the Pittsburgh meeting.

[2] Numbers appearing in parentheses correspond to those given to references listed at the end of this paper.

egg white which will whip when mixed with water, the proteins must be altered before drying or the dried material must be aged for several months at room temperature or for several hours at an elevated temperature, 80 to 127°C.(176 to 260°F.) (9). The whipping quality may be imparted to dried egg white by bacterial fermentation previous to drying (10). This alteration is due to proteolytic enzyme action of the bacteria. A number of years ago some investigators found that the same result could be accomplished by partial digestion with added trypsin (11), and that the time and the amount of trypsin required were reduced if the natural antienzyme of the white was destroyed by taking advantage of the destructive effect of time, temperature, and high pH (10.5-11.5). Tray-dried egg white requires less alteration previous to drying than does spray-dried white. It was found that the whipping quality of altered tray-dried egg white was destroyed by fine grinding in a ball mill (11). The tray-dried white is often sold in a granular form erroneously called albumin crystals.

WHIPPING QUALITY OF NATURAL EGG WHITE

Only recently the whipping quality of natural egg white has attracted investigators (12). It was stated that the thick white did not whip to give so large a foam as did the thin white and did not make so large an angel food cake (13). This statement is in line with the old household custom of holding for a few days eggs to be used in whipping. The failure of the thick white to give as large a foam as the thin white is due to the use of inefficient egg beaters (14), which shove the thick white to one side and do not disintegrate it. Thick white, homogenized, gives as large a volume as does thin white; fermented and enzyme-treated dried white gives a larger volume of whip than does the natural egg white (11). Freezing natural egg white does not affect its whipping qualities. So far as the author knows, no one has succeeded in making a satisfactory angel food cake from spray-dried egg white.

CHEMICAL EVIDENCE OF QUALITY OF EGGS

A progressive increase in loosely bound ammonia in the yolk (15), water-soluble or inorganic phosphorus (16) and water content of the yolk (17), and of the acid number of the fat of the yolk (18) have been observed as eggs are held. It has been suggested that the fluorescence of the shell and white changes with age (19). The solids in both yolk and white may be calculated from refractive index measurements (11, 20).

In the white no decrease in glucose (11, 21), change in optical rotation (11, 22), or definite increase in simpler protein fractions

occurs with age in the uninfected egg until it enters the inedible grade (11). Chemical methods, together with bacterial count, odor, and taste, are used in the determination of the quality of frozen eggs. Marked chemical changes are found in eggs which are badly infected with microörganisms, but in these cases the bacterial determinations are often of more significance than chemical tests. The bacterial contamination of held shell eggs usually occurs in the yolk and can be determined by simply streaking on an agar plate (11) a loop of material taken from the yolk, and incubating the plate. The bacteria are usually present either in large numbers or not at all.

Physical methods for measuring quality of shell eggs are usually much more satisfactory, simple, and rapid than chemical methods.

PHYSICAL EVIDENCE OF QUALITY OBTAINED BY CANDLING

It is assumed that an ideal fresh egg is the desired objective in quality, and that eggs should be graded in terms of departure from this condition. Quality as determined by candling is based primarily on the size of the air cell and secondarily on the visibility and movement of the yolk (23). The size of the air cell depends on the time, temperature, humidity, and circulation of the air. The visibility of the yolk depends upon its color (23, 24), the condition and amount of the apparent thick white (23), the color of the shell, and the characteristics of the candling equipment (25). The ordinary consumer does not candle the egg but breaks it into a dish. Candling is the best method available for rating unbroken eggs but is not always reliable for indicating the quality of the opened egg (23, 24, 26). Eggs are bought and sold on the basis of candling quality but are consumed on the basis of opened egg appearance and flavor. Egg grades are expressed in terms of candling quality. This places the emphasis on a false objective and has led to such devices as controlled feeding to produce light-colored yolks to render them less visible in candling. Efforts should be made to change the objective and statement of the grades from that of eggs which candle well to that of eggs which are desirable when they are opened.

PHYSICAL EVIDENCE OF QUALITY OBTAINED BY OPENING

A high-quality egg should have a good flavor, upstanding yolk of uniform color, and 70 to 80 per cent of upstanding apparent thick white. Egg flavor is affected by the feed (27) and individuality (28) of the hen, presence of microörganisms, natural chemical breakdown (29, 30), and the absorption of flavors from the surroundings (30).

The progressive flattening and weakening (31) of the yolk is associated with the passage of water from the white to the yolk, owing to osmotic forces (32, 33), and can be followed very simply and expressed numerically as the yolk index, obtained by dividing the height by the width (33). The yolk index is one of the best indications of the degree of mistreatment of the egg, and in general is independent of the original amount and condition of the thick white which varies greatly even in fresh eggs. The yolk may become mottled because of the entrance of water and because of localized mechanical disturbances which expose different portions of the layers of the yolk (34).

Beginning at the shell, the white is made up of the following layers: outer thin, structural thick white sac, inner thin, and yolk envelope thick white (35). The structural thick white sac is composed of a number of layers which can be seen if magnified about 100 diameters (11) or which may be brought into prominence by special treatment (36). The thick white sac and the white contained in it are called the apparent thick white. It is the apparent thick white which one sees as the jelly-like material around the yolk when an egg of good quality is opened. The condition (35), albumin index, or height (37) of the thick white is a very important quality factor. As the egg is mistreated the apparent thick white becomes flabby and flat, finally the sac breaks, the thin white contained in it runs out, and eventually the sac itself disintegrates.

The presence of mucin in the thick white has been indicated (38). Since thick white can be changed to thin white by simple mechanical disintegration which was not expected to set free bound water, no appreciable difference in the amount of water bound by the thick and thin white was expected. Although the experimental evidence on this point is contradictory (39), the most recent study indicates that there is no difference. The factors influencing the amount and condition of the white have been studied recently by a number of investigators (40).

Photographs of eggs showing the effect of time and temperature on deterioration are shown (Fig. 1).

FACTORS INVOLVED IN PRESERVATION OF EGGS

The successful preservation of eggs depends on the following factors: quality of the eggs placed in storage, sterility of the egg contents, prevention of luxuriant growth of microörganisms on the shell, pH of the contents, prevention of evaporation, prevention of absorption of flavors, and temperature.

All successful methods involve these factors to varying degrees, but in many instances all conditions are not optimum and the true

interrelation of all factors to give the best storage conditions has never been determined. The general relation between four important egg-quality characteristics and the storage variables which influence deterioration is illustrated graphically (Fig. 2). Three types of successful methods and the factors involved follow:

Water-glass Immersion: The alkalinity of the solution prevents the growth of microörganisms, and the water prevents evaporation and the escape of carbon dioxide and thus maintains a low pH.

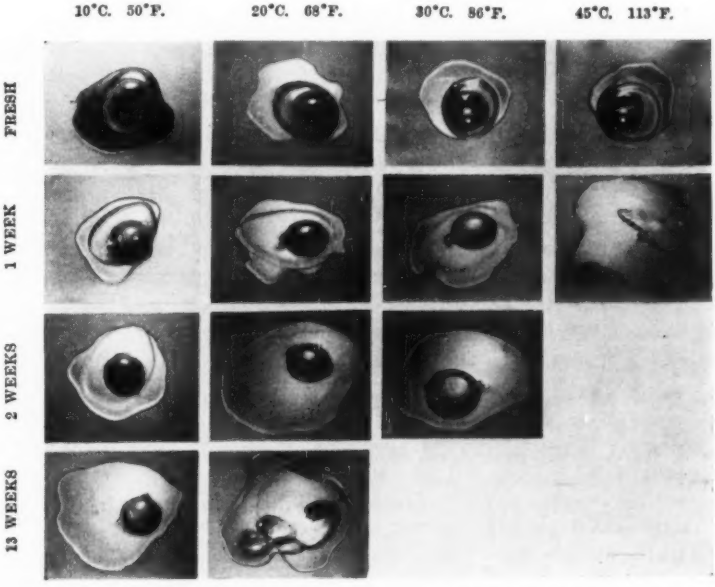


FIG. 1. Effect of time and temperature on deterioration of eggs. Upstanding yolks and thick jelly-like whites surrounding the yolks are desirable.

Oiling the Shell: The shell is partially sealed, which retards evaporation and the escape of carbon dioxide, thus maintaining a low pH. Chance of subsequent infection with microörganisms is lessened.

Ordinary Cold Storage: The natural weakening and disintegration of the colloidal structure of the white and passage of water into the yolk is retarded at low temperatures, but the humidity must be controlled to prevent excessive loss in weight and the luxuriant growth of microörganisms on the shell. Much of the carbon dioxide is lost; yet the lowered rate of loss at low temperature and the fact that the eggs are confined make it possible to keep them for some months.

QUALITY OF ORIGINAL EGGS

The quality of eggs taken from a cold-storage warehouse is influenced most by the quality of eggs which enter it. On the whole, hens lay eggs of good quality, but often an appreciable part of the useful life of the egg has passed before it enters storage (41). The ability of hens to lay eggs of high quality is an inherited factor (42) probably influenced by feed or physiological condition of the hen. As the laying season advances, the freshly laid eggs of some hens become poorer in quality, possibly because of the depletion of some essential factor.

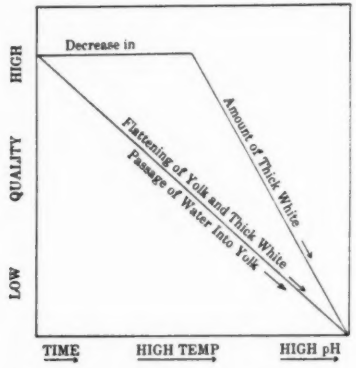


FIG. 2. General rate of change of four important quality factors as due to any one of the three important causes of deterioration: time, high temperature, or high pH.

Eggs laid in the early spring are generally of better quality at the end of the storage season than are eggs laid in the late spring or summer. This difference is due to two causes: The eggs laid in the early part of the storage season are of better quality (43); also since the weather of early spring is cold, the eggs are cooled rapidly (44) and arrive at the storage warehouse with less deterioration.

Porosity of the Shell: The pores are of two types—the large pores which are actual holes in the shell that can be seen with a low-power microscope, and the small pores which are protein-filled channels between the crystals of calcium carbonate (45, 46). There are about 15 of the former and several thousand of the latter per egg. The greater the number of large pores the faster the egg loses weight (45). The small pores have little direct effect on the loss in weight unless the eggs are stored over a strong dehydrating agent, such as concentrated sulfuric acid (11, 47). The thicker the shell (45, 48) the more slowly the egg loses weight (11, 45). Rarely the yolk is infected

when the egg is laid, infection after the egg is laid occurs through the large pores.

Eggs With Soiled Shells: Not over one to two per cent of eggs properly cared for are infected with microörganisms (11, 49, 50). Upon this fact rests the successful storage of eggs and the production of frozen egg products of low bacterial content. The number of infected eggs is much greater if the shells become soiled (50, 51). If the soiled eggs are washed, the evidence is removed that a high degree of infection is to be expected; and consequently if stored, a great number of the eggs rot. Washed eggs can be detected by the absence of potassium chloride on the shell, and scraped eggs can be detected by staining with rosaniline hydrochloride (52). Filtered ultra-violet light (black light) can be used to detect by fluorescence the stain left on the shell by some types of dirt after the dirt visible in normal light has been removed by washing (11).

CONTROL OF FLAVOR

The so-called "storage flavor" may be due to absorption of flavors from the surroundings (53, 54), which can be largely prevented by choosing fillers, flats, pads, and cases which impart a minimum of flavor to the eggs (54). Warehouse men some years ago recognized that the flavor acquired from strawboard fillers and flats become more pronounced the higher the humidity of the storage room. Great improvements have since been made by some of the manufacturers of strawboard fillers. These improved materials impart no more flavor than do some of the spruce fillers, and the flavor absorbed is not increased appreciably at higher humidities (54). The odor of the storage room is often controlled by adding ozone (56).

Flavors develop within the egg because of natural chemical changes. The development of flavor of this type is markedly accelerated by high pH values, and consequently its development can be retarded by pH control (54, 55).

RELATIVE HUMIDITY

Since commercially the grade of eggs is determined largely by the size of the air cell as seen in candling, the warehouse man makes every effort to prevent the evaporation of water from the eggs, by maintaining as high a humidity as possible in the storage rooms, and yet prevent the luxuriant growth of mold on the eggs. Mold grows luxuriantly if the relative humidity at the shell surface is 96 per cent or higher (57). A slight white mold growth called "whiskers" occurs at humidities ranging from 90 to 94 per cent. This growth is often used as an indication that the humidity is being maintained

at a desirably high level (58); although if the storage period is abnormally extended, infection of the egg contents results (57).

The safe humidity as determined in the aisle of the storage room depends on many factors, such as the velocity of air circulation, stacking of the cases, moisture in the wood and fillers of the cases, and fluctuations in temperature. The humidity in the aisle usually falls within the limits of 80 to 93 per cent (59). The trend is toward relative humidities above 90 per cent. The egg is in osmotic equilibrium with air of a relative humidity of about 99.5 per cent.

VELOCITY OF AIR

The rate of evaporation of water from an exposed egg is independent of air movement (11, 60) except at extremely low velocities. An imperceptible movement eliminates the gradient in the atmosphere so that the humidity of the air at the shell evaporating surface is the same as the humidity at a distance. When this end is achieved, further increases in velocity produce no further increase in the rate of loss in weight. The forced circulation of air which is maintained in many egg-storage rooms tends to prevent localized points of high humidity where molds can grow luxuriantly and permits a higher humidity in the aisle. An increase in humidity reduces the evaporation of eggs in the more exposed portions of the cases but has relatively little effect on eggs in the center of the cases. At a constant humidity, an increase in the velocity of air in the room has little effect on the rate of evaporation of the exposed eggs comprising the outer layers, but it does cause the eggs in the interior of the case to lose weight at a faster rate (11). This is because the eggs in the filler cells in the center of the cases have a humidity gradient in the air for some distance from the shell surface. Increasing air velocity tends to remove the gradient in the air from eggs further within the case.

SHELL TREATING OF EGGS

The use of over a hundred materials for shell treating of eggs has been patented, ranging from cactus juice, rubber, and cellulose compounds to oil coating. Oil coating, used by Ben Franklin and in Holland in 1807, has proved the most successful and the cheapest. The chief characteristic required of a successful coating is close adhesion to prevent mold growth between the coating and the shell. (In some cases even oil treatment leads to mold growth in large pores.) The coating should prevent evaporation and the escape of carbon dioxide and should be odorless, tasteless, and invisible. The effectiveness of oil in preventing the escape of moisture depends on the properties of the oil, degree of penetration, structure of the shell, and humidity and temperature of storage (11, 61). Under various

conditions the loss in weight is reduced from one-fifth to one-thirtieth of that of unoiled eggs.

Case Liners: In order to reduce the loss in weight of unoiled eggs to be held for relatively short periods, the eggs in each half of the case are sealed in moisture-proof bags or liners which fit snugly inside of the case and around the eggs and fillers. Eggs will mold if stored in this type of packing, because the humidity will reach a level at which molds will grow (62). Molds will also grow if the eggs are individually wrapped in moisture-proof materials.

Storage in an Inert Gas: In parts of Europe eggs are stored in sealed containers in which the oxygen is replaced by an inert gas. Nitrogen and carbon dioxide are usually selected (63). This prevents the growth of mold at high humidities.

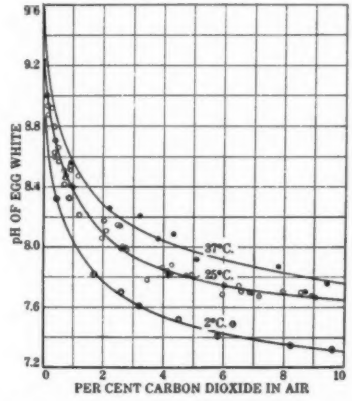


FIG. 3. The pH of egg white when in equilibrium with air containing various amounts of carbon dioxide.

At the time of being laid, the egg is in approximate carbon dioxide equilibrium with the tissues of the hen. The pH of the yolk is about 6.0 and the white 7.6 or less (64). The white contains a bicarbonate buffer which at room temperature is in equilibrium with an atmosphere containing about 10 per cent of carbon dioxide. The carbon dioxide escapes fairly readily from a well-ventilated egg so that after a few days the pH rises to about 9.6. This rise in pH can be prevented by oil-treating the shell or by storing the egg in an atmosphere containing the equilibrium amount of carbon dioxide.

The equilibrium amount of carbon dioxide which the air must contain to hold the white at various pH values is given (Fig. 3). The actual volume of carbon dioxide in the white varies (Fig. 4), but at

the same carbon dioxide tension, irrespective of the amount of carbon dioxide which the white takes up, the pH value is approximately constant.

While the fact that the yolk has a higher osmotic pressure than does the white accounts for the passage of water from the white to the yolk, the pH of the white markedly influences the rate of this passage, because, of course, the pH of the liquids bathing a membrane

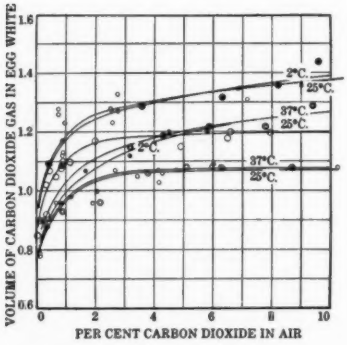


FIG. 4. Cubic centimeters of carbon dioxide in each cubic centimeter of egg white when in equilibrium with air containing various amounts of carbon dioxide.

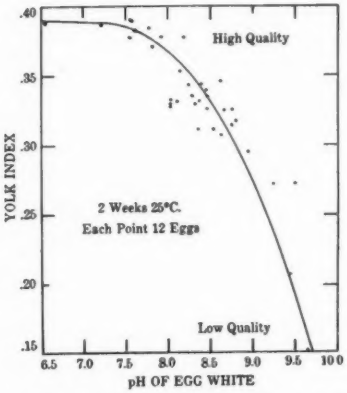


FIG. 5. Effect of the pH of egg white, maintained with carbon dioxide in the air, on yolk index (ratio of height divided by width).

(yolk membrane in this instance) markedly influences the rate of passage of the solvent under osmotic gradient. This is shown clearly by the effect of pH of the white on the yolk index (Fig. 5). The lowering of the yolk index under most conditions is directly related to the passage of water into the yolk (33). The effect of pH on the condition of the thick white is shown (Figs. 6 and 7). The relation between the pH and the amount of outer thin white is more complex, showing a minimum in the zone 8.0 to 8.5 (Fig. 8). This minimum is due to the interrelation of two factors—the effect of low pH values which tend to shrink the thick white gel around the yolk, apparently causing some extrusion of the inner thin white, and the effect of high pH which tends to disintegrate the thick white gel.

The relation between concentration of carbon dioxide in the air and various quality factors when eggs were stored for about 8.5 months at —1°C.(30.2°F.) is presented (Fig. 9). The higher the pH the more rapidly the whites become yellow during storage (29).

CAUSE OF STERILE EGG DETERIORATION

A study of the deterioration of eggs was begun at Cornell in 1925 with the assumption that the breakdown of the white was due to enzyme action (11). However, the investigators were unable to detect by chemical means, alterations in the true protein fractions until all of the white had become completely watery (11). The main role was assigned to the known hydrolytic effect of water catalyzed by hydroxyl ions (high pH) operating first to simplify the colloidal structure (67, 66). The enzyme theory has recently been revived

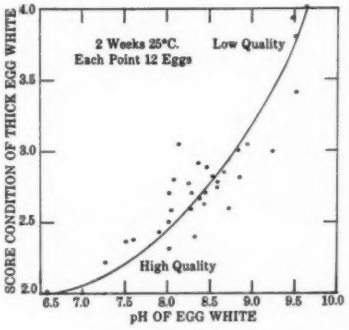
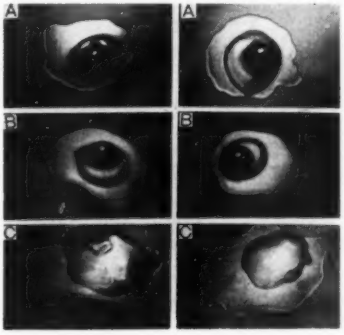


FIG. 6. Effect of pH of egg white, maintained with carbon dioxide in air, on the jelly-like condition of thick egg white. The higher the score the more undesirable and flabby the thick white.



A=FRESH.
B=4 WEEKS 86°F., 25% CARBON DIOXIDE.
C=4 WEEKS 86°F., NO CARBON DIOXIDE.

FIG. 7. Effect of low pH, maintained with carbon dioxide in air, in preventing development of flabbiness or breakdown of the jelly-like thick egg white and weakening of the yolk.

and accepted by one group of investigators (68) and questioned by another (69).

It is possible that some one will perform original additional experiments which will make it possible to decide definitely between these two explanations, but at the present time several of the behaviors of eggs are not in agreement with the enzyme theory of shell-egg deterioration. The thick white in which the enzyme is supposed to be located keeps best at the pH which is optimum for the action of the enzyme found (69). The fact that trypsin when injected into an egg causes a breakdown of the egg contents does not prove that trypsin is a constituent of egg white. Trypsin was used commercially a number of years ago to prepare egg white for drying (11). It has been stated that thick white would autolyze, presumably in a reasonable time. A definite study of this point failed to show autolysis (69), and it is

shown here that eggs can be kept at pH near 7.6 for four weeks at 30°C.(86°F.) in the unopened condition without showing any appreciable breakdown of the thick white or appreciable change in the yolk (Fig. 7).

Direct proof of protein cleavage was not presented; casein, not egg white, was used as a substrate. It is claimed by some workers

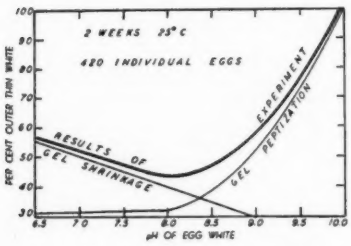


FIG. 8. Effect of pH, maintained with carbon dioxide in air, on amount of outer thin egg white. Below pH 8.0-8.5 amount of outer thin egg white increases because of shrinkage of the gel structure. Above this pH zone the amount of thin egg white increases because of disintegration or peptization of the gel.

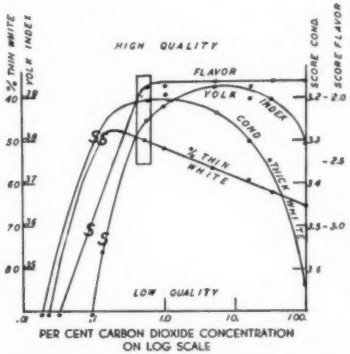


FIG. 9. Relation between pH of egg white, maintained by carbon dioxide in air, and the quality factors of eggs stored (1930) for 8.5 months at —1°C. The points in the rectangle were obtained from eggs stored in a room containing 15 carloads, .55% of carbon dioxide being maintained in the atmosphere. The S marks represent the values for normal storage. Each point represents 4 dozen eggs. (Sharp and Stewart.)

that trypsin is the enzyme present (68); while other investigators believe the evidence of trypsin based on enterokinase action is inconclusive, that the enzyme has more of the properties of erepsin, and that it will not attack the natural egg white (69). The actual shrinkage or decrease in amount of the thick white cannot be taken as proof of enzyme action, because gel-like structures often shrink or swell with age and changes in environment. Specifically, the thick-white structure of eggs can be made to shrink by altering the pH.

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INFLUENCE OF CALCIUM IN COOKING WATER UPON MINERAL CONTENT OF VEGETABLES

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(Received for publication, September 1, 1937)

Little consideration has been given to the effect which the presence of calcium in cooking water may have upon mineral changes in vegetables during cooking. One instance of a gain, however, has been reported. This was by Ziegelmayer (1931), who found that potatoes boiled in very hard water (56.7 degrees hardness) contained 4.58 times as much calcium as raw potatoes, and in medium hard water (6.9 degrees) 1.07 times as much. The present study was initiated to see if similar behavior could be expected of other vegetables. Green beans, asparagus, spinach, peas, and carrots were used.

EXPERIMENTAL PROCEDURE

A sufficiently large amount of each vegetable for the entire experiment was purchased on the retail market, prepared for cooking, mixed as well as possible, and then divided into four four-serving portions. One of these was set aside to be analyzed raw, the second was cooked in Chicago city water, the third in calcium chloride solution, and the remaining one in distilled water. The weights of the four-serving portions and the details of preparation and cooking are given by Halliday and Noble (1933). The vegetable samples and the cooking waters were oxidized with nitric and perchloric acids, and the clear solution so obtained analyzed for calcium by the Shohl and Pedley (1922) modification of the McCrudden (1911-12) method and for phosphorus by the Fiske and Subbarrow (1925) colorimetric one. The phosphorus determinations, while not directly concerned with the problem at hand, were included to see if the large amounts of calcium present in certain of the cooking waters would affect the phosphorus losses.

DISCUSSION OF RESULTS

All of the vegetables except carrots, when cooked in either Chicago city water or calcium chloride solution, contained more calcium than when raw, but when cooked in distilled water, contained less (Table 1). Thus green beans, which showed the least change of any of the vegetables, contained 48 mg. of calcium per 100 grams of raw sample, 51 mg. per equivalent quantity after cooking in Chicago city water,

TABLE 1

Calcium Content of Vegetables Cooked in Liquids of Different Calcium Concentrations

Vegetable	Calcium per quantity of vegetable equivalent to 100 grams raw				Calcium per liter of sample of Chicago water used	Calcium per liter of calcium chloride solution	Loss (—) or gain (+) during cooking		
	Raw	Cooked in distilled water	Cooked in Chicago water	Cooked in calcium chloride solution			Distilled water	Chicago water	$CaCl_2$ solution
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*pct.*	*pct.*	*pct.*
Green beans	48	46	51	51	38	76	—4	+6	+6
Carrots (sample 2)	42	34	35	39	34	78	—19	—17	—7
Asparagus	19	16	21	24	33	83	—16	+11	+26
Spinach leaves	60	58	65	71	36	83	—3	+8	+18
Peas (sample 2)	20	18	23	28	38	105	—10	+15	+40
Peas (sample 1)	28	23	28	40	30	158	—18	0	+43
Carrots (sample 1)	32	25	26	25	36	15	—22	—19	—22

the same amount after cooking in a calcium chloride solution of a little more than twice the calcium concentration of the Chicago water, and 46 after cooking in distilled water. Corresponding results for asparagus, which showed the greatest change of any of the vegetables

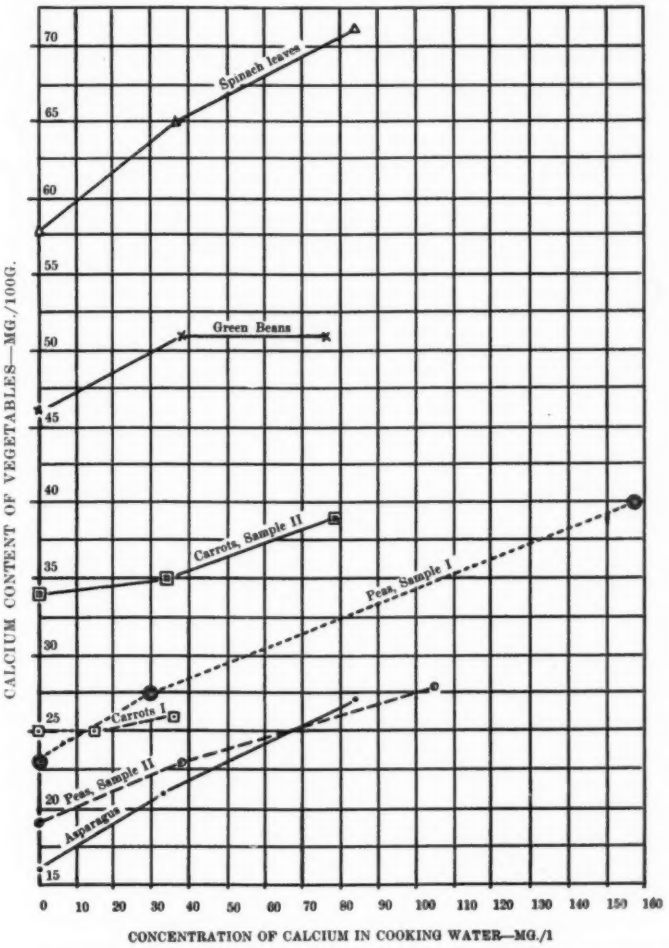

FIG. 1. Relation between the calcium content of raw and boiled vegetables and the calcium concentration of the cooking water.

cooked in similar concentrations of calcium, were 19, 21, 24, and 16. These figures represent for the beans a gain of six per cent in Chicago water and in calcium chloride solution and a loss of four per cent in

distilled water, and for the asparagus gains of 11 and 26 and a loss of 16 per cent. Carrots, even though suffering losses in all of the cooking liquids, did lose less when calcium was present than when it was not.

Thus it seems that increasing the calcium concentration of the cooking liquid may be expected to produce a calcium content in the cooked vegetable larger certainly than that of a sample cooked in distilled water and in many instances than of the raw vegetable. The amount which can be introduced in this fashion (even if one should want to) is limited, however, for the quantities taken up by any one vegetable become relatively smaller as the concentration increases.

TABLE 2

Phosphorus Content of Vegetables Cooked in Liquids of Different Calcium Concentrations

Vegetable	Phosphorus per quantity of vegetable equivalent to 100 grams raw				Loss during cooking		
	Raw	Cooked in distilled water	Cooked in Chicago city water [1]	Cooked in $CaCl_2$ solution [1]	Distilled water	Chicago water	Calcium chloride solution
	mg.	*mg.*	*mg.*	*mg.*	*pct.*	*pct.*	*pct.*
Green beans	46	41	41	39	11	11	15
Carrots (sample 2)	27	22	20	22	19	26	19
Asparagus	68	57	56	59	16	18	13
Spinach leaves	37	21	23	21	43	38	43
Peas (sample 2)	99	77	83	76	22	16	23
Carrots (sample 1)	22	17	16	16	23	27	27

[1] For the calcium concentration see Table 1.

This situation is shown graphically (Fig. 1), the calcium content of the vegetables being plotted against the calcium concentration of the cooking liquids. As will be noted the curve for each vegetable tends to flatten as it approaches the higher concentrations, some more rapidly than others. Beans, for example, had apparently almost reached their maximum value in the calcium chloride solution used; but spinach, asparagus, and peas had not, in spite of the fact that both samples of peas were cooked in more concentrated calcium solutions than any of the other vegetables.

Physical changes, such as toughening, which may accompany the cooking of vegetables in hard water, have not been considered because interest has centered primarily in the effect of the calcium concentrations not far from that of Chicago city water. In this medium vegetables become tender a little faster than in distilled water.

The losses in phosphorus were apparently unaffected by the calcium concentration of the cooking liquid (Table 2), since there is

very little difference among them for any one vegetable and since such differences as do exist are not consistently higher or lower for any of the cooking liquids.

SUMMARY

Vegetables cooked in liquids of different calcium concentrations contained without exception more calcium than when cooked in distilled water, and with one exception, more than when raw. They suffered approximately equal losses of phosphorus during cooking.

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THERMAL DEATH-TIME STUDIES OF *OOSPORA LACTIS*

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(Received for publication, September 1, 1937)

Microörganisms of various types are playing greater and greater rôles in problems relating to food preservation. The canning and meat packing industries are founded upon efforts to control bacterial development. In the dairy industry various microörganisms bring about both desirable and undesirable changes. Among the undesirable organisms in milk and dairy products are fungi of which *Oospora lactis* is a typical example. This fungus has been used in recent years as an indicator of undesirable conditions, and it is now necessary to know much more about it. Since several dairy products are prepared from pasteurized milk or cream, it is necessary that more be known about its heat resistance. The results reported in this paper involve that practical problem.

HISTORICAL REVIEW

Interest in Fungi Imperfecti or intermediary molds started at the beginning of the nineteenth century and has continued to the present day. Link (1809) isolated many of these organisms and placed them in a genus, *Oidium*. In 1851 Fresenius isolated an organism with the same general characteristics from milk. He described the colonies as creamy-white, circular, flat, with concentric zones. Aerial hyphae were seldom produced; if so, they were short and moniliform. The mycelium was septate and continuous at first, soon breaking into individual cells; older growths showed a torula-like condition at the edge of the culture. *Oidium lactis* was the name given to it because of its peculiar association with sour cream and milk. It remained for Saccardo (1886) to place it quite properly in the genus *Oospora*, described and named by Wallroth (1833). Being very similar in morphology and physiology, these two generic names have been used interchangeably, *Oidium* being the more widely employed. However, as pointed out by Hammer (1935), these genera are separated mainly on the basis of pathogenicity for animals. *Oidium* is the name usually given the pathogenic, while *Oospora* is used for the non-pathogenic or saprophytic varieties.

Lindau (1900) included within the tribe *Oosporae* both genera *Oidium* and *Oospora*. Similarity of the two genera is marked, and

in many respects the characteristics of one closely paralleled those of the other. Until more investigations are carried out to permit clearer differentiation between the two genera, their relationship will continue to be hazy. Upon the basis of present information it seems advisable to use the name, *Oospora lactis*. Sixty-one cultures isolated from butter by Macy and Gibson (1937) apparently showed considerable diversity in characteristics.

Interest in the organism and its thermal resistance was of minor significance until interest in production of higher grade butter and cream developed through the agencies of pasteurization and cold storage. Among the first to determine thermal resistance of *Oospora lactis* was Ocallaghan (1901), who reported that fishy flavor of butter was caused by *Oidium lactis*. The organisms causing the fishy flavor can be readily destroyed at a pasteurization temperature of 74°C. (165.2°F.) for 30 minutes. Epstein (1902) reported that the organism was commonly present in cream and butter. To free butter and cream of its presence in order to carry on further experimentation, these products must be completely sterilized. Thom and Ayres (1916) found the holding process of pasteurization to be efficient for killing many yeasts and molds, among which was *Oospora lactis*. It survived pasteurization temperatures of 51.7°C.(125°F.) for 30 minutes but was completely killed at a temperature of 54.4°C.(130°F.) and higher temperatures.

Combs and Eckles (1917) discussed the relationship of *Oidium lactis* and *Penicillium* to the keeping qualities of butter. Both molds were pasteurized in cream at different temperatures. Keeping quality of the resulting butter was greatly improved when cream was pasteurized at 62.8°C.(145°F.). Pasteurization at 71.1°C.(160°F.) was even more effective, while at 100°C.(212°F.) the deterioration owing to presence of the mold seemed to be entirely removed.

Hunziker, *et al.* (1917) reported that the vat method of pasteurization at 62.8°C.(145°F.) was 99.94 per cent efficient in destroying yeasts, molds, and other microörganisms. Tables of results further demonstrated that 20 minutes is the minimum length of exposure at which reasonably high germ-killing efficiency may be expected. Repeating vat pasteurization using sour cream, Hammer (1914) secured results at 60 to 62.8°C.(140 to 145°F.) for 30 minutes. Twenty minutes sometimes left large numbers of living organisms although the percentage killed was high.

Nicholls (1919) recommended the holding method of pasteurization instead of the flash method. He found that *Oospora lactis* was able to survive brief exposures to temperatures of 82.2°C.(180°F.) and higher.

Henrici (1930) stated that *Oospora lactis* is a common and widespread mold, extraordinarily resistant to heat and antiseptics, which grows everywhere that lactic acid is present.

Parfitt and Sorenson (1935) isolated eight different strains of *Oospora lactis*. One strain had an optimum growth temperature at 37°C.(98.6°F.) instead of the usual 25°C.(77°F.). It was suggested that this strain might have thermal resistance which is considerably higher than the thermal resistance of the remaining seven strains.

Macy and Gibson (1937) reported that three cultures of *Oospora lactis* resisted heating at 57°C.(134.6°F.) for 30 minutes, one at 60°C. (140°F.) for the same period. None survived 62.8°C.(145°F.) for 30 minutes. It would seem, therefore, that careful pasteurization ought to destroy *Oospora lactis* in products which allow reasonable heat penetration.

EXPERIMENTAL PROCEDURE

In order to determine accurately heat resistance of *Oospora lactis* in thick cream a large group of cultures was isolated. They came from samples of commercial cream arriving at large commercial stations located near the University of Illinois. The samples were collected prior to pasteurization of the cream, great care being taken to select samples from wide geographical distribution. The states from which cream samples were received were Illinois, Indiana, Kentucky, Ohio, Nebraska, Missouri, and Arkansas.

Several methods of collection were attempted. The most satisfactory was sampling the cream with a large tablespoon and transferring it directly to a sterile sample bottle.

Sterile pipettes were used (whenever possible) to transfer a portion of the cream samples to a standard sterile petri plate. Because of thickness and viscosity, it was difficult to handle some specimens with a pipette. These were transferred in small lumps by means of a sterile spatula. When isolating the organisms, efforts at quantitative results were not made. Nevertheless, the amount of cream transferred had to be large enough to give a number of *Oospora lactis* colonies distributed over an area that would allow convenient selection of single colonies. Following transfer of cream to the petri plates, six to ten cubic centimeters of melted wort agar 40 to 45°C.(104 to 113°F.) were poured into the plates and the contents thoroughly mixed. The plates were inverted and allowed to incubate at room temperature for 24 to 60 hours before typical *Oospora lactis* colonies were selected.

The literature lacks detailed morphological and physiological descriptions of *Oospora lactis*. We therefore followed those published by Henrici (1930), Jensen (1912), and Thom (1906).

Using the available descriptions, Parfitt and Sorenson (1935), after studying the large group of cultures, attempted to group *Oospora lactis* strains according to differences in morphology and physiology. They reported eight strains with different colony structure, cell shape, action on litmus milk, growth curves, caseolysis, lipolysis, proteolysis, and other physiological characteristics.

The procedure for determining the heat resistance of the organism used in this work was based upon that usually used. Small sealed tubes (eight millimeters in diameter) containing the organism and the medium was completely submerged in a water bath held at a constant temperature for a certain period of time.

TABLE 1

Results of Pasteurization of Oospora lactis

	Temperature	Time of incubation	Cultures tested	Positive	Negative	Positive controls
Coffee cream	°F.	*hrs.*				*pct.*
(22% butterfat)	145	6	224	0	224	100
Coffee cream						
(22% butterfat)	145	48	64	0	64	100
Heavy Cream No. 1						
(40% butterfat)	145	18	48	0	48	100
Coffee cream						
(22% butterfat)	135	24	144	8	136	100
Coffee cream						
(22% butterfat)	125	24	24	24	0	100

From many cultures, 224 were chosen as typical *Oospora lactis* according to morphological and physiological characteristics reported in the literature. Preparation for determination of thermal resistance was carried out by transferring the organism from the stock wort agar slant to ten cubic centimeters of coffee cream sterilized by the flash method of seven minutes in an autoclave at 18 pounds pressure and 118°C.(245°F.). The cream was cooled immediately so that its physical and chemical makeup would not be changed by the heat. Transfers were then made directly from the cream to the thermal death-time tubes after the cream had incubated at 25°C.(77°F.) for varying lengths of time.

The thick inoculated cream which was used in these experiments was delivered to the bottom of the thermal death-time tubes by means of capillary pipettes. The tubes were sealed and were then ready for the water bath. After being heated 30 minutes the tubes were quickly removed and plunged into cold water to stop the action of the heat.

Thermal death-time tubes containing the pasteurized cream were opened individually by filing one end open. The thickness of the cream caused some trouble when attempts were made to remove it. Finally, however, it was forced out by striking the tube vigorously. One-tenth and one-fifth cubic centimeter amounts were used because only qualitative results were desired. Wort agar was selected as the recovery medium. After the poured agar had hardened sufficiently, the plates were inverted and incubated at room temperature, 22 to 25°C.(70 to 77°F.), for 60 to 150 hours.

If growth of *Oospora lactis* was definitely confirmed by re-isolation of a pure culture of the original culture, the results were recorded positive (+). If growth of *Oospora lactis* could not be definitely confirmed by re-isolation of the original culture, the results were recorded negative (—).

The 224 organisms were allowed to grow in the cream for approximately six hours, transfers being made to thermal death-time tubes and pasteurized for 30 minutes, at 62.8°C.(145°F.). This temperature was selected because it approximates that used in actual practice. All of the 224 cultures thus pasteurized were destroyed when heated in cream under the conditions just described. The 20 controls were not destroyed and gave heavy growth covering the entire surface of the wort agar.

Sixty-four of these original 224 cultures remained at room temperature until 60 hours had elapsed. Appearing upon the surface of the cream was a one-eighth to one-fourth inch mat or felt-like covering which was characteristically wrinkled. This was thoroughly broken and distributed throughout the cream before the usual sample was removed for subsequent pasteurization. The 20 controls again proved the presence of the original culture qualitatively. Even though that concentration of the organism in the cream had increased significantly, however, it could not be detected in the results because all of the 60 cultures were killed at 62.8°C., as shown by the sterile wort agar after 150 hours of incubation.

To more nearly duplicate cream used in making butter, a thick heavy cream scoring No. 1 (40 per cent butterfat) was employed in the following experiment. Forty-eight strains of the organism were incubated at room temperature for 18 hours to insure satisfactory concentration of *Oospora lactis*. Pasteurization at 62.8°C. destroyed all of them. Heavy growth obtained in all of the 12 control wort agar plates showed that viable *Oospora lactis* was present.

Up to the present, the investigation showed that *Oospora lactis* was unable to withstand a pasteurization temperature of 62.8°C. for a period of 30 minutes in cream. To find a temperature which the

organism could resist for 30 minutes, 144 of the original cultures of *Oospora lactis* were transferred to sterile coffee cream. These strains were exposed as described above to a temperature of 57.2°C.(135°F.) for 30 minutes. All but eight were killed, as evidenced by lack of growth on the plates prepared from heated cream. These eight were subjected to the same conditions of the preceding experiment and seven were killed. The remaining culture, No. 194, was re-pasteurized twice with the same conditions and was recovered both times. It was a slightly more heat-resistant culture than the other 143 organisms. The 12 controls again yielded positive results in proving the presence of the organism. Attempts were next made to locate the temperature at which all cultures survived 30 minutes' exposure. Using 24-hour slants of 24 of the original cultures, the pasteurization was repeated with coffee cream at 51.7°C.(125°F.). All of the cultures resisted this temperature as was shown by a luxuriant growth of *Oospora lactis* upon the wort agar.

Having learned the heat resistance of a large number of strains of *Oospora lactis,* it was decided to study still further several strains which seemed to stand out by themselves in resistance to heat in cream. The technic was quite like that already described.

In preparing a given culture for the test, growth of one 24- to 48-hour wort agar slant was suspended with the aid of a sterilized loop in three cubic centimeters of a sterile one-fifteenth molar phosphate solution (Sorenson mixture of Na_2HPO_4 and KH_2PO_4 having a pH of 7.0). Removal of clumps which might interfere with the rapid penetration of heat to all the cells was affected by the filtration of the suspension through sterile cheese cloth into a sterile tube. Enough additional phosphate solution was then added to bring the total volume to ten cubic centimeters.

One cubic centimeter of each suspension being tested was placed in as many tubes as required for the particular experiment. The open ends of the tubes were then closed in the torch flame, after which they were cooled in ice water.

After all the tubes had been sealed and cooled, the baskets of tubes were placed in the bath so that the tubes were completely submerged. The temperature of the bath was half a degree above the necessary actual temperature so that the first cooling effect would not cause the temperature to fall below the temperature desired. Greater variation of the temperature through $\pm$ .5°F. was not allowed. Baskets were removed at predetermined periods to detect viability of *Oospora lactis* cells.

Following removal of the tubes and subsequent cooling, the ends were filed, broken off, and the entire contents were poured into a

sterile petri plate. Melted wort agar 40 to 45°C.(104 to 113°F.) was added to each petri plate followed by a 60- to 150-hour incubation period at room temperatures.

When growth of *Oospora lactis* colonies occurred on the plates, the results were recorded positive (+); when growth of *Oospora lactis* did not occur on the plates, the results were recorded negative (—).

The results are believed to show fairly accurately the thermal resistance of strains of *Oospora lactis* commonly encountered in the dairy industry (Table 2). All cultures came from cream samples with the exception of Culture D, obtained from Professor Parfitt. Results are given for temperatures at 54.4, 57.2, 60, and 62.8°C.(130, 135, 140, and 145°F.).

SUMMARY

Two hundred and twenty-four cultures of *Oospora lactis* were isolated from samples of cream from a wide geographical area. None of these cultures was able to resist pasteurization in cream at 62.8°C. (145°F.) for 30 minutes. One hundred and forty-four cultures were exposed in cream to 57.2°C.(135°F.) for 30 minutes. Eight cultures successfully resisted this temperature. Pasteurization at 51.7°C. (125°F.) did not kill any of the cultures used in this investigation.

Temperatures ranging from 125 to 145°F. at 5°F. intervals were used to determine thermal resistance of nine cultures in phosphate solution. One fairly heat-resistant strain withstood pasteurization for 30 minutes at all temperatures used up to 20 minutes at 145°F.

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TABLE 2

Results of Thermal Death-Time Studies

Cultures	Temperature	Length of exposure in minutes					
		5	10	15	20	25	30
	°F.						
94	130	+	+	+	—	+	—
	135	—	—	—	—	—	—
	140	—	—	—	—	—	—
	145	—	—	—	—	—	—
97	130	+	+	+	+	+	+
	135	+	—	—	—	—	—
	140	—	—	—	—	—	—
	145	—	—	—	—	—	—
146	130	+	+	+	+	+	+
	135	+	—	—	—	—	—
	140	—	—	—	—	—	—
	145	—	—	—	—	—	—
154	130	+	+	+	+	+	+
	135	+	—	—	—	—	—
	140	—	—	—	—	—	—
	145	—	—	—	—	—	—
194	130	+	+	+	+	+	+
	135	+	+	+	+	+	+
	140	+	—	—	—	—	—
	145	±(?)	—	—	—	—	—
221	130	+	+	+	—	—	—
	135	+	—	—	—	—	—
	140	—	—	—	—	—	—
	145	—	—	—	—	—	—
225	130	+	+	+	—	—	—
	135	+	—	—	—	—	—
	140	—	—	—	—	—	—
	145	—	—	—	—	—	—
237	130	+	+	+	+	+	+
	135	+	—	—	—	—	—
	140	—	—	—	—	—	—
	145	—	—	—	—	—	—
D	130	+	+	+	+	+	+
	135	+	+	+	+	+	+
	140	+	+	+	+	+	+
	145	+	+	+	+	—	—

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TYPES AND SURVIVAL OF SOME MICROÖRGANISMS IN FROZEN-PACK PEAS, BEANS, AND SWEET CORN GROWN IN THE EAST [1]

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(Received for publication, September 18, 1937)

INTRODUCTION

Many workers have pointed out that, although the microbial content of fresh foods is greatly reduced in numbers, these foods are not completely sterilized by storing them at freezing temperatures for long periods. The literature on this subject has been very well reviewed by James (1932), Wallace and Tanner (1933), and Berry and Magoon (1934).

Since freezing storage of food does not bring about sterilization, the only way to avoid possible development of dangerously high microbial content or contamination with harmful organisms in handling or storage of frozen food is to control the microbial content of this food during its preparation and storage and until it is eaten. In other words frozen food must be treated with at least as much care in observing proper sanitary measures to keep it clean as fresh food. To provide a fair basis for judging whether or not the microbial content of frozen fruits and vegetables is within reasonable bounds it is necessary to know something of the types and numbers of microörganisms ordinarily present on these foods in their fresh state and the normal reduction in numbers and types to be expected under the conditions imposed by frozen pack.

Some workers in this field have limited their studies to the microbial flora of fruits and vegetables after the food was frozen, e.g., Prescott (1932) and Lochhead and Jones (1936). Such studies are valuable in supplying knowledge of the number and character of the organisms which survive freezing and storage but they give no idea of the original microbial population of the raw material or of the degree to which this population is reduced by freezing treatment. Since the original load of organisms on the raw material will vary widely with its previous history, it must be known before we can form any idea of the extent to which freezing processes tend to partially sterilize frozen products. Berry (1937) has included determina-

[1] Food Research Division Contribution No. 341.

tion of the microbial content of fresh vegetables in his report on western-grown, frozen-pack spinach, kale, asparagus, peas, and corn; and Smart (1934a) gives the microbial content of fresh as well as frozen strawberries, and also of fresh as well as frozen mushrooms (1934b). Smart and Brunstetter (1936) have given microbial content of one variety of lima beans in both fresh and frozen conditions and have made the same sort of study on spinach and kale (1937).

No reports have appeared in the literature on the microbial content of eastern-grown green beans or sweet corn, giving the numbers and types of microörganisms found on the fresh vegetables and also after storage at freezing temperature. This report is based on such a study of 14 varieties of green and wax beans and 35 varieties and strains of sweet corn and also includes a study of eight varieties of lima beans in both the fresh state and after storage, the latter supplementing the work of Smart and Brunstetter (1936), who reported on only one variety of lima beans. The microbial content of 18 varieties of frozen peas is also given. The microbiological studies were started after the peas were packed and frozen so that, unfortunately, the work on this vegetable is incomplete in so far as the bacterial flora of the raw material is concerned.

MATERIAL USED

During recent years Caldwell and his coworkers have carried on experiments to determine the varietal suitability of eastern-grown fruits and vegetables for frozen pack under various conditions of treatment and temperature. In 1935 an extensive packing program was carried out by these workers for the purpose of summarizing previous observations on frozen-pack peas, green and wax beans, sweet corn, and lima beans. Their experiments are now complete and have been reported by Caldwell, Lutz, Culpepper, and Moon (1936); Caldwell, Lutz, and Moon (1936); Moon, Caldwell, Lutz, and Culpepper (1936); and Moon, Caldwell, and Lutz (1936).

Microbiological studies on this material form the basis of this report. At all times during the preparation and cutting of the packs the utmost consideration was shown the writer by workers in charge of the experiment by facilitating taking samples for microbiological analysis that would be representative of the material going into the packs. Without this consideration it would have been impossible to sample adequately the large amount of material packed during the season.

The vegetables used in the experimental frozen packs were grown at Arlington Experiment Farm, Rosslyn, Virginia. While rainfall was not plentiful during the growing season of many of the vege-

tables, ample water was supplied them by means of the excellent irrigation system at the experimental farm. Other weather conditions were favorable throughout the growing season and also at harvest time, except that at the time the lima beans were harvested heavy rains fell, causing the pods to become spattered with mud.

Insect pests and fungous diseases were kept at a minimum by constant, careful attention. The vegetables, because of the excellent care given them to supply the requirements for optimum growth and the favorable weather conditions, approached the finest quality that it is possible to produce in this region. The varieties of vegetables chosen for trial in frozen pack were those already successfully grown and popular for various reasons in several localities in the East.

PACKING METHODS

Peas: The eighteen varieties of peas tested in this experiment were harvested by hand in the early morning, brought to the laboratory, run through a pea sheller, and graded by use of standard sieves. After being graded they were thoroughly washed in running tap water. Small lots of the peas were then enclosed in cheesecloth sacks and dipped into rapidly boiling water. The volume of water used in scalding was sufficient to prevent much change in its temperature during the scalding treatment. The small sizes ($^{8}/_{32}$ or $^{9}/_{32}$ inch in diameter) were scalded one minute; the intermediate sizes were given two minutes' scalding; while the larger peas ($^{13}/_{32}$ or $^{14}/_{32}$ inch in diameter) were scalded three minutes.

After the scalding treatment the peas were cooled in running tap water to about 12.8 to 18.3°C.(55 to 65°F.), following which they were packed in one-pound paraffined paper cups or No. 2 sanitary enameled tin cans. Part of the packs were frozen without added liquid, while part had two per cent brine added to them. Cups were closed and cans were then sealed and placed in cold storage at —9.4°C.(15°F.). When the packs were frozen solid they were stored at —17.8°C.(0°F.) until they were cut for examination after five to seven months.

Green and Wax Beans: Fourteen varieties, 12 of green and two of wax beans, were treated and packed in much the same way as described for peas. The small beans were packed whole after the ends were cut off and after being scalded in boiling water for two minutes and cooled in running tap water. The older beans were broken or cut into inch-length pieces, were scalded three minutes, and cooled. Half the packs were filled with two per cent brine and half were frozen without added liquid, employing both paraffined

paper cups and sanitary tin cans. These packs were frozen and stored in the same way as described for peas.

Sweet Corn: Thirty-five varieties and strains of sweet corn were studied to determine their comparative suitability to frozen pack. The harvesting was done in early morning, and treating and packing were carried out as rapidly as possible to insure a maximum amount of sugar and flavor in the frozen product. The corn was husked, silked, trimmed when necessary, and washed, entirely by hand. It was then enclosed in cheesecloth sacks and scalded four minutes in boiling water. Following this, it was cooled to the temperature of tap water, 12.8 to 18.3°C.(55 to 65°F.), and packed in paper cups or tin cans. Some of the corn was packed as whole ears in five- and ten-pound containers both in sweetened brine and without liquid. The pack without added liquid was in part unwrapped; in part the individual ears were enclosed in a wrapping of either cellophane, waxed paper, or aluminum foil. Part of the corn that was cut from the cob was cut as whole grain and part as cream style. Some of this corn was packed in sweetened brine and some without it. Both paper cups and tin cans were used as containers. All packs were frozen and stored as described for peas.

Lima Beans: Six bush varieties and two pole varieties were included in the study of lima beans. The beans were harvested, shelled, and graded by hand.

The small sizes were scalded two minutes, the intermediate three minutes, and the large sizes four minutes. Following the scalding treatment the beans were cooled in tap water and packed, frozen, and stored as described for peas.

CUTTING AND EXAMINATION OF FROZEN VEGETABLES

After a storage period of from five to seven months, the frozen peas, green and wax beans, sweet corn, and lima beans were cut and graded in the raw state for color, texture, and general appearance. The vegetables were then cooked and graded for color after cooking, texture, flavor, and general table quality. All the grading was done by the same group of people, all of whom were experienced in judging frozen fruits and vegetables. Where varieties of vegetables best suited to frozen pack were used, the quality of the cooked product was excellent and in many instances better than the fresh vegetables available in the retail market.

This description of the packing methods used in the experimental packs of frozen vegetables has been given to provide a foundation for understanding the microbiological data. Complete reports of the

varietal studies may be found in the four references to the work of Caldwell, Lutz, Culpepper, and Moon.

METHODS USED IN MICROBIOLOGICAL ANALYSIS

The first samples for microbiological analysis were taken after the fresh vegetables were washed and prepared for scalding. However, a few tests were made on the vegetables as they came from the field to determine the efficiency of the washing methods. Water used for washing and brine used as a packing medium were frequently examined during this experiment to determine microbial content.

The next samples were taken from the packing table after the vegetables had been scalded and cooled. Final sampling was done at the end of the storage period which was from five to seven months in length.

Samples consisted of 100 grams of the material to be tested, selected from various parts of the mass so that the sample would represent the mass as accurately as possible. The material so selected was weighed out into sterile jars and 100 c.c. of sterile tap water added. Following thorough mixing, dilutions were made from the mixture and plates poured, using beef agar adjusted to pH 7.0 for bacteria and wort agar adjusted to pH 4.5 for molds and yeasts.

The beef agar plates were incubated for two days at room temperature, 29°C.(84.2°F.), while the wort agar plates were allowed to incubate for five days. The predominating types of bacteria, yeasts, and molds were transferred to agar slants and run through the prescribed tests for species identification. The names of the species are given in the tables where there was little or no doubt concerning their identity, otherwise only the genus name is given.

MICROBIAL CONTENT OF FRESH AND SCALDED VEGETABLES

The microbial content of fresh vegetables has been studied by various authors for purposes other than frozen-pack investigations. Prescott and Underwood (1898) made a very complete study of the microbial content of fresh sweet corn for the purpose of determining the proper processing time for canned corn. Kurk (1918) investigated the microbial content of green vegetables (lettuce, onions, celery, radishes, and watercress) from the public-health point of view. James (1925a and 1925b) has reported on the microbial content of fresh sweet corn and fresh head lettuce. Tanner (1934) has given an excellent account of the public-health aspects of fresh and frozen vegetables, and Berry (1937) has given data on the microbial content of a number of fresh western-grown vegetables. All investigators,

whether their interest is centered around the frozen packing of vegetables or some other phase of the subject, have found wide variation in the microbial content of different lots of vegetables.

In the experiment now being considered the factor causing the greatest variation in microbial content of the fresh vegetables was ear-worm infestation in sweet corn. Ears of corn which were free from worms averaged for one variety a microbial count of 100,000 per gram and for another variety 500,000. Ears of corn from which it was necessary to trim even small parts which were being consumed by ear worms had counts of 10,000,000 per gram for the first variety mentioned above and 7,000,000 per gram for the second. These counts are not unusually high according to James (1925a).

The ear worm, by cutting holes through the husk, makes possible the introduction into the ear of air- and water-borne microörganisms as well as those on his body and in his intestinal tract. The grains of corn, after the worm has cut them open, afford an abundant food supply for the growth of these microörganisms. Rains and dew spread the inoculum and the food material down between the rows of grains over the entire ear. This sticky or gummy material is not easily removed by washing. Washing and trimming of ear-worm-infested sweet corn intended for frozen pack must be very carefully done.

The amount of rainfall prior to harvesting is another factor which greatly affects the microbial content of fresh vegetables. This was pointed out by Smart and Brunstetter (1936), who found that lima beans picked during dry weather averaged a count of 20,000 microörganisms per gram for one lot and 80,000 per gram for another lot, while beans harvested following a rain and still wet when brought to the laboratory had a count of 10,000,000 per gram of material. The lima beans used in the experiment reported here were harvested during a rainy season and the counts are rather high, averaging 424,000 per gram.

Any lack of care in preparing vegetables for frozen pack and any delay in cooling and freezing the material is sure to be reflected in a higher microbial content of the material so treated.

Apparently all fresh fruits and vegetables have a fairly distinctive microflora, for although the same genera of microörganisms may appear on plates from samples of different fruits and vegetables, there is always a different proportion of each genus and usually different species or strains of the types present. In general Achromobacter, Pseudomonas, and Flavobacterium are the genera which appeared most plentifully on the plates poured from the fresh vegetables used in this study. Various molds are also present. After the

scalding treatment Bacillus, Flavobacterium, and Sarcina were the predominating genera present. Yeasts and molds are also plentiful on all plates. The lowest, highest, and average microbial counts per gram on the vegetables, both fresh and scalded, are given (Table 1) as well as the names of the types of microörganisms isolated (Table 4).

The figures emphasize the wide variation in the microbial content found in the different lots of fresh vegetables used in these studies (Table 1). Some of the possible factors causing these variations have already been discussed. The reduction in microbial content by scalding is also subject to wide variation.

TABLE 1

Microbial Content of Fresh and Scalded Vegetables

Vegetable	Description of sample	Highest count	Lowest count	Average of all counts
		per gm.	*per gm.*	*per gm.*
Green and wax beans	Fresh beans	1,000,000	35,000	800,000
	Scalded beans (2, 3, and 4 min.[1] at 212°F.)	800,000	400	47,000 (a reduction of 94.13%)
Sweet corn	Fresh corn	10,000,000	100,000	3,000,000
	Scalded corn (4 min at 212°F.)	3,000,000	10,000	375,000 (a reduction of 87.5%)
Lima beans	Fresh beans	1,000,000	40,000	424,000
	Scalded beans (2, 3, and 4 min.[1] at 212°F.)	150,000	1,000	90,000 (a reduction of 78.8%)

[1] Scalding time varies with maturity of vegetables. (Data on scalded vegetables in the table represents all lots regardless of length of scalding.)

It can be seen that sweet corn, because of its higher microbial content, may present to the packer a greater problem of microbial control than either green or lima beans. The greater sugar content of sweet corn may be responsible for this, although it has been already pointed out that ear-worm infestation has a great deal to do with high counts on the fresh corn.

Scalded vegetables with their collapsed cells and higher water content furnish a better medium for rapid microbial growth than do fresh vegetables within certain time limits. Therefore, in order to avoid high counts and possible spoilage it is necessary that scalded vegetables be cooled promptly and kept cool until frozen. Actual freezing should take place as rapidly as possible after harvesting vegetables in order to insure a product with a low microbial content.

MICROBIAL CONTENT OF FROZEN VEGETABLES AFTER FIVE TO SEVEN MONTHS' STORAGE

The effect of low temperatures on the growth and survival of microörganisms has been the subject of much investigation. In connection with work on the microbiological problems connected with frozen-pack foods, Berry (1933) reports that very low temperatures are likely to be less lethal for the microörganisms contained in frozen fruits and vegetables than moderately low temperatures.

Beard and Cleary (1932) believe that the germicidal effect of hydrogen ion concentration becomes weaker as the temperature is lowered and recommend the same care in regulations and inspections for frozen acid foods as for non-acid.

Osmotic pressure, varying water and sugar content, and many other factors have also been considered as possible explanations for

TABLE 2

Microbial Content of Frozen Vegetables After Five to Seven Months' Storage

Product	Highest count	Lowest count	Average of all counts
	per gm.	*per gm.*	*per gm.*
Peas	20,000	100	1,200
Green and wax beans	10,000	50	900
Sweet corn	1,000,000	200	160,000
Lima beans	90,000	150	13,000

the rather erratic behavior of microörganisms when stored at low temperatures.

All investigators—Loehhead and Jones (1936), Prescott (1932), and Berry (1937)—have agreed that the microbial content of fruits and vegetables is greatly reduced by storage at freezing temperatures but that these foods are not sterile even after long storage periods; hence frozen-pack food must be handled with not only the same care as fresh foods before they are frozen but with even greater care, and they must be more quickly utilized after thawing than is the case with fresh foods just received from the market.

Although Prescott and Geer (1936) point out that there is no well-authenticated case of food poisoning from frozen foods that have been properly handled, they warn, "It must be emphasized that all foods of this type, subject to the possible presence of the organisms (disease producing), should be prepared in the most strictly sanitary manner with a minimum delay between harvesting and freezing."

The microbial content of the frozen vegetables studied in this experiment is subject to wide variation. This variation in numbers

might have been less noticeable had the vegetables been stored for longer periods. The highest, lowest, and average microbial counts on the vegetables studied (Table 2) and the percentage of reduction in the microbial content brought about by scalding, freezing, and storage for five to seven months (Table 3) are given here.

The average of all counts on the various frozen vegetables show that a high microbial content is avoidable if care is taken to keep high-quality products clean and cool during packing and to make the period between harvesting and freezing as short as possible.

The greatest number of samples for bacteriological examination was taken from the one-pound containers, but enough samples from the vegetables packed in the five- and ten-pound containers were

TABLE 3

Reduction in Microbial Content of Vegetables After Scalding and After Five to Seven Months' Storage at Freezing Temperature

Product	Microbial content (av.) fresh vegetables	Microbial content (av.) scalded vegetables	Microbial content (av.) frozen vegetables after 5 to 7 months' storage	Reduction in microbial content of vegetables owing to scalding and storage
	per gm.	*per gm.*	*per gm.*	*pct.*
Green and wax beans	800,000	47,000	900	99.8
Sweet corn	3,000,000	375,000	160,000	94.6
Lima beans	424,000	90,000	13,000	97.2

examined to determine any possible differences which might occur in the microbial content of vegetables treated alike except for the size of container in which they were frozen. There was found to be no consistent differences in the microbial content of the vegetables whether frozen in large or small containers. In a few instances the vegetables in the large containers had a lower microbial content than those of like treatment packed in one-pound boxes. This lower count is probably the reflection of a somewhat greater care on the part of the packers to see that the larger containers were put in the most advantageous positions in the freezing room in order to insure rapid cooling.

Frozen sweet corn under the conditions of this experiment had the highest microbial count per gram and the smallest percentage of reduction caused by freezing storage. Sweet corn has a higher pH value than either green or lima beans and also a higher sugar content. It may be that these factors were, at least in part, responsible for the greater resistance of the microörganisms present to the killing effect of the storage temperature.

In general, yeasts, molds, and various species of lactobacillus were more numerous in frozen sweet corn and peas than were the other microbial forms present (Table 4). In frozen green beans and lima beans Bacillus, Flavobacterium, Sarcina, and Micrococci were more numerous than the molds and yeasts, although the latter were present in nearly all samples.

It will be noticed that among the microörganisms repeatedly isolated from the frozen-pack products there are in some instances many genera listed (Table 4) that do not appear in the list of types isolated from the fresh vegetables. It may be that some of the microörganisms were present on the fresh vegetables but were unable to grow on the plates because they were crowded out by faster growing types. However, some of these organisms were undoubtedly introduced during the preparation of the packs by means of contaminated blanching and cooling water, utensils and containers, as well as the hands of the packers.

The microbial flora of frozen vegetables, in regard to both types and numbers present, may be influenced by the hydrogen ion concentration and sugar content of the fresh product, although these are probably only two of the many factors involved.

Because the microbial content of some frozen-pack vegetables may be at times much higher than desirable even under the best conditions of packing and storing, these foods should be thoroughly boiled before they are eaten. To preserve the maximum amount of quality and to prevent any chance of spoilage, frozen vegetables should not be allowed to defrost before cooking is started. They should be placed in boiling water while they are still solidly frozen and the cooking time calculated from the time the thawed mass begins to boil.

Where varieties of vegetables well adapted to frozen pack were used, the quality of the cooked product was excellent. High microbial content in some of the packs appeared to have had some effect in producing off-flavor and loss of color in the product. In other packs where the microbial content was equally high there seemed to be no falling off in quality of the cooked product. More work will have to be done in this connection before any definite conclusions can be drawn.

It must be kept in mind continually, in preparing and using frozen-pack foods, that the same rules for sanitary control which have been found necessary in handling fresh foods must be observed in handling frozen foods, which are essentially still fresh foods.

SUMMARY

The results of a bacteriological examination of eastern-grown vegetables in the fresh state and in frozen pack are presented. Eighteen varieties of frozen peas were studied, but data such as counts and types of microörganisms on the fresh peas are lacking. Complete studies were made, both on fresh vegetables and again after scalding and freezing, on 14 varieties of green and wax beans, 35 varieties of sweet corn, and eight varieties of lima beans.

The fresh vegetables examined were found to have a high microbial content (Table 1) which varied considerably in different lots. Two factors causing much higher counts than average in the vegetables used in this experiment were ear-worm infestation in sweet corn and heavy rainfall prior to the harvesting of many of the vegetables.

The types of microörganisms present on fresh vegetables (Table 4) were principally soil types of bacteria, yeasts, and molds, but the predominating types were found to be somewhat different for each of the products studied.

The pretreatment of scalding vegetables and the freezing and storage at —17.8°C.(0°F.) for five to seven months reduced the average microbial content of green beans 99.8 per cent; lima beans, 97.2 per cent; and sweet corn, 94.6 per cent. In spite of this average reduction in microbial content, some of the packs were found to contain up to one million microörganisms per gram (in sweet corn). The types persisting after storage treatment were principally soil types which are destroyed by boiling the vegetables.

High counts in fresh sweet corn may be associated with ear-worm infestation. High counts in frozen sweet corn may be the result in part of an initially higher number of organisms present and in part of a greater degree of protection which sweet corn affords the microörganisms that contaminate it against the killing effects of low temperatures than is afforded by either green beans or lima beans. It is suggested that the sugar content of sweet corn, which is higher than that of the other two products, may have some part in this protective action and also that the high pH values of sweet corn may be a factor involved.

Frozen foods, which are still essentially fresh foods, must be subjected to at least the same care in observing proper sanitary control during their preparation and storage as has been found necessary in handling fresh foods. They must be cooked immediately upon thawing or defrosting.

TABLE 4

Microörganisms Repeatedly Isolated From Fresh and Frozen Vegetables

	From fresh and blanched vegetables	From frozen vegetables
PEAS	(Work begun after peas were packed.)	Bacteria: Azotobacter sp. *Bacillus megatherium* DeBary [1] *Bacillus mycoides* Flügge *Bacillus vulgatus* Trevisan Flavobacterium sp.[1] Lactobacillus sp.[1] *Pseudomonas fluorescens* Migula [1] Sarcina sp. Yeasts: Saccharomyces sp.[1] Torula sp. (red)[1] Torula sp. (brown)[1] Molds: Aspergillus sp.[1] Monilia sp.[1] Penicillium sp.[1] Rhizopus sp.
GREEN AND WAX BEANS	Bacteria: Achromobacter sp.[1] *Bacillus albolactis* Migula [1] *Bacillus cereus* Frankland and Frankland *Bacillus mycoides* Flügge Flavobacterium sp.[1] Phytomonas sp. *Pseudomonas fluorescens* Migula Sarcina sp.[1] Streptococcus sp. Yeasts: Endomyces sp.[1] Torula sp. (red) Molds: Cladosporium sp.[1] Monilia sp. Penicillium sp.[1] Rhizopus sp.	Bacteria: Achromobacter sp.[1] Azotobacter sp. *Bacillus megatherium* DeBary [1] *Bacillus mycoides* Flügge [1] *Bacillus simplex* Gottheil [1] Flavobacterium sp.[1] *Pseudomonas fluorescens* Migula Sarcina sp. Spirillum sp. Yeasts: Torula sp. (pink) Molds: Monilia sp.[1] Penicillium sp. Rhizopus sp.

[1] Predominating types.

[1] Predominating types.

TABLE 4 *(Concluded)*

SWEET CORN	Bacteria: Achromobacter sp. Aerobacter sp.[1] Bacillus sp.[1] Lactobacillus sp.[1] Micrococcus sp. *Pseudomonas fluorescens* Migula [1] Sarcina sp. Serratia sp. Yeasts: Endomyces sp. Mycoderma sp. Saccharomyces sp.[1] Torula sp. Molds: Fusarium sp. Monilia sp.[1] Oidium sp.[1] Penicillium sp. Rhizopus sp.	Bacteria: Aerobacter sp. Bacillus sp.[1] Lactobacillus sp.[1] Micrococcus sp.[1] Sarcina sp.[1] Serratia sp.[1] Streptococcus sp. Yeasts: Saccharomyces sp.[1] Mycoderma sp. Molds: Monilia sp.[1] Oidium sp.[1] Penicillium sp. Rhizopus sp.
LIMA BEANS	Bacteria: Achromobacter sp.[1] *Aerobacter aerogenes* (Kruse) Beijerinck *Bacillus cereus* Frankland and Frankland Flavobacterium sp.[1] Micrococcus sp. *Pseudomonas fluorescens* Migula [1] Sarcina sp.[1] Streptococcus sp. Yeasts: Torula sp. Molds: Cladosporium sp. Dematium sp.[1] Monilia sp. Penicillium sp.[1]	Bacteria: *Achromobacter radiobacter* (Beijerinck and Van Delden) comb. nov.[1] Aerobacter sp. *Bacillus parvus* Neide. Flavobacterium sp.[1] *Pseudomonas fluorescens* Migula [1] Sarcina sp. Yeasts: Saccharomyces sp. Molds: Dematium sp. Monilia sp.[1] Penicillium sp.

[1] Predominating types.

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MINERAL CONSTITUENTS OF HONEY
II. PHOSPHOROUS, CALCIUM, MAGNESIUM

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(Received for publication, September 18, 1937)

Data on the composition of the ash of honey are neither extensive nor searching. Approximately sixteen communications relative to some —and in a few cases all—of its twelve known inorganic constituents have appeared in the literature within the last three decades, a period in which the trend has been either toward the substitution of quantitative for such qualitative data on the mineral components of a food as may have been then extant, or the expansion of the former if it existed. Relevant to this communication are the following: Van Dine and Thompson (1908), to whom credit for priority in reporting on the calcium and magnesium content of honey probably belongs; and, latterly, reports of Fehlmann (1912); Sundberg and Lundgren (1930); Jewell (1931); and Svoboda (1930 and 1933), with respect to the three elements in question; and those of Elser (1925, 1926, 1928) on phosphorus and calcium.

Early recognized by Hilger (1901) as a factor contributive to the alkalinity of the soluble ash constituents of honey, phosphorus was first reported by him (1904) to exist here as salts of calcium and magnesium admixed with the dextrins precipitated by methyl alcohol. Eventually Young (1908) furnished confirmation for this observation in that he microscopically identified these salts as phosphates. More recently Paine, Gertler, and Lothrop (1934) found it among the colloidal constituents of this food. Interest in the extent to which it occurs in honey, as reflected by the published information thereon by Erlenmeyer and von Planta (1874), Mader (1890), and Villaret (1893), apparently antedates the others of this group. This is not because of the nutritional virtues of this element but rather because of the promise which it seemed to offer as a diagnostic agent in the detection of added foreign syrups.

The objectives sought by those to whom reference has been made in the foregoing compilation are diverse. Aside from a desire to extend the borders of information on the mineral matter of honey not only for academic reasons but also for the purpose of obtaining diagnostic data for differentiating between genuine and adulterated honeys (Elser) or floral and honey-dew honeys (Van Dine and Thompson), the inorganic constituents of this food have also been

studied in their relation to floral source (Nottbohm), to "dwindling" of the bee colony (Jewell), to predisposition to foul brood (Sundberg and Lundgren), and to geographical distribution of nectar source (Fehlmann).

Our interest lay in a desire to determine whether any qualitative relationship might exist between degree of pigmentation of honey and the content of all three of the aforementioned elements although we were not unmindful of a statement by Hehner (1885) that the proportion of phosphoric anhydride stands in direct relation to the color of genuine honey. Inasmuch as his conclusions were based upon the examination of but four samples whose degree of pigmentation was expressed in qualitative terms, it bid fair to be worth while to repeat his experiments on a larger and more representative number of honeys in the light of modern technics of color measurement and description and a sensitive colorimetric micro-method for the determination of phosphorus. Then, too, the desirability of obtaining comparable data on honeys grown in the United States, for none, apparently, have been gathered since the few which were reported from an insular possession in 1908, gave the problem a two-fold objective.

To this end there were secured from growers in California, Colorado, Georgia, Illinois, New York, Texas, Wisconsin, Wyoming, and the Canal Zone 35 honeys, most of which were in comb sections from which the wax was subsequently separated in the laboratory. Representatives of the whole gamut of color classification which is used in the merchandising of honey (Sechrist), from the so-called water-white to dark, were among the group which had been assembled for this study. Included herein were honeys whose predominant floral sources were, respectively, alfalfa, several kinds of clover, cotton blossoms, orange blossoms, white sage, wild asters, gallberry, tupelo gum, thistle, Mexican clover, titi, and buckwheat.

EXPERIMENTAL PROCEDURE

Ashing of Samples: Samples of the order of magnitude of 250 grams were first carbonized in large platinum dishes in the presence of pure olive oil, added for the purpose of controlling the frothing which ensues when saccharine substances are burned, after which the operation was completed in an electrically heated furnace whose temperature was kept at a dull redness. This procedure was modified, however, when it affected samples whose phosphorus content was to be determined because preliminary experiments on some 14 honeys of diverse color classification had shown that losses of this element of 40 parts per million may be expected if to the material

under examination there is not added a basic substance before it is burned. Parenthetically, it may be remarked that, with the exception of Nottbohm (1927), all who have reported data on the phosphorus content of honey had apparently either overlooked the possibility of a loss of this element in this instance or, cognizant of it, deemed it an insignificant source of error. The physical effect of this treatment was the formation of a white, fluffy ash.

Determination of Acid-Insoluble Matter: This determination was carried out as for silica—the resulting data are hereinafter referred to as such—except that the final step, volatilization of this element as the tetrafluoride, was omitted. The hydrochloric acid (1 + 1) solution of the ash was twice evaporated to dryness after filtration. It was then diluted to 100 c.c. of which aliquot portions were later used for the determination of calcium, magnesium, and phosphorus, respectively.

Determination of Phosphorus: The Truog and Meyer (1929) modification of the ceruleomolybdate method of Denigès (1920) was used for the determination of phosphorus in aliquot portions of ash solutions containing from .73 to 1.45 grams which had been prepared from sample weights one hundred times as large. The accuracy of the method in its application to the problem in hand was tested in five control determinations on 50 grams of pure sucrose to which five mg. of phosphorus as KH_2PO_4 had been added. The mean deviation in recovery of this element was $\pm$.048 mg., or .96 per cent.

Determination of Calcium: Calcium was precipitated with a saturated oxalic acid solution in the presence of ammonium hydroxide, a procedure critically studied and recommended by Breazeale (1920). It was then determined volumetrically. Recovery experiments on four controls containing both calcium and magnesium (2.5 mg. Ca) showed a mean deviation of $\pm$.03 mg., or 1.2 per cent. Sample weights for this series of analyses varied from 43 to 156 grams.

Determination of Magnesium: Magnesium was precipitated with 8-hydroxyquinoline by the Hough-Ficklen modification (1930) of the method of Strebinger and Reif (1929). The accuracy of the method, as well as the influence of the personal equation, was checked in four trials against a solution containing one mg. of magnesium. A mean deviation of $\pm$.012 mg., numerically equivalent to the same per cent, was noted.

Determination of Alkalinity of Ash: Three fairly high-ash representatives of different floral sources and color classifications were chosen on which to make that series of determinations which, by long practice, is associated with this term. Since the procedures herein involved are so well known, they hardly require description.

TABLE 1

Ash, Silica, Phosphorus, Calcium, and Magnesium Content of Honey

Classification	Samples	Ash	Silica		Phosphorus		Calcium		Magnesium	
			Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.
Light honeys		*pct.*								
Water-white	3	.045	4.83	24	7.23	31	10.83	50	4.39	18
White	11	.096	2.90	21	5.51	39	6.72	47	2.46	20
Average	....	.070	3.86	22	6.37	35	8.77	49	3.42	19
Dark honeys										
Light amber	12	.125	3.42	37	4.00	42	5.05	60	2.59	32
Amber	3	.221	2.22	46	2.92	52	3.03	60	1.76	36
Dark	6	.120	2.97	26	4.10	48	2.63	33	2.96	38
Average	....	.155	2.87	36	3.67	47	3.57	51	2.77	35

TABLE 2

Variation in Silica, Phosphorus, Calcium, and Magnesium Content of Light and Dark Floral Honeys

Constituent	Light honeys				Dark honeys			
	Minimum		Maximum		Minimum		Maximum	
	Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.	Pct. of ash	Mg. per kg.
Silica (SiO_2)	1.60	14	7.07	36	1.03	13	5.82	72
Phosphorus	1.03	23	9.55	50	0.84	27	6.67	58
Calcium	3.54	23	13.00	68	0.46	5	7.30	266
Magnesium	1.00	11	9.24	56	0.66	7	11.47	126

RESULTS

Data for each of the 35 honeys which were analyzed during the course of this investigation have not been made a part of this record because degree of pigmentation rather than floral source was primarily the point of departure herein involved, a course consistent with the current merchandising practice, for no requirement as to the latter is made in the United States grades for honey except that covered by the phrase "freedom from damage caused by objectionable flavor." To that end, following the determination of the position of each honey on the Pfund scale (Sechrist), the data (Table 1) were averaged for each of the five major color groups, viz., water-white, white, light amber, amber, and dark. Next, all data were more or less arbitrarily grouped again into two larger divisions, light and dark, as typifying, in a qualitative sense, the most obvious differences in pigmentation. The immediate effect of this segregation is that the first group is comprised of those honeys whose predominating floral sources are clover, alfalfa, white sage, orange flowers, and cotton blossoms; the second buckwheat, wild asters and other late-blooming flowers, and those, except cotton, originating in the South, such as gallberry, tupelo gum, Mexican clover, and titi. Finally, since this compilation does not reveal the limits within which each of the elements in question occurs in each main division, the variations in content thereof have been made the subject of a separate one (Table 2).

DISCUSSION

It is again evident, as once before pointed out by Schuette and Remy (1932) that mineral content and degree of pigmentation apparently bear some relationship to each other, for the dark honeys in contrast to the light-colored ones herein examined were found to contain, on the average, the larger quantities of the inorganic constituents. This relationship in respect to these two main color divisions is, perforce, qualitative. It awaits a quantitative interpretation until such time as it may be possible to assemble for analysis a series of honeys each of which is the exclusive product of but one floral source. But not, perhaps, without some significance is the fact that, in this instance, the difference in total ash content between these color divisions is represented by a 2.2-to-1 ratio in favor of the dark honeys; that, similarly, magnesium stands next in line in this respect, the ratio being 1.86:1; silica and phosphorus follow with a 1.6- and 1.4-fold increase, respectively, of the deeply pigmented honeys over those less so; whereas in the case of calcium the difference is but slight, or 1.04:1.

When the data (Table 1) are given graphic form (Fig. 1), an interesting situation reveals itself with respect to the elements of phosphorus and magnesium inasmuch as both show an almost parallel increase in concentration with a rise in position on the Pfund color scale. This increase holds particularly for the latter in all the color

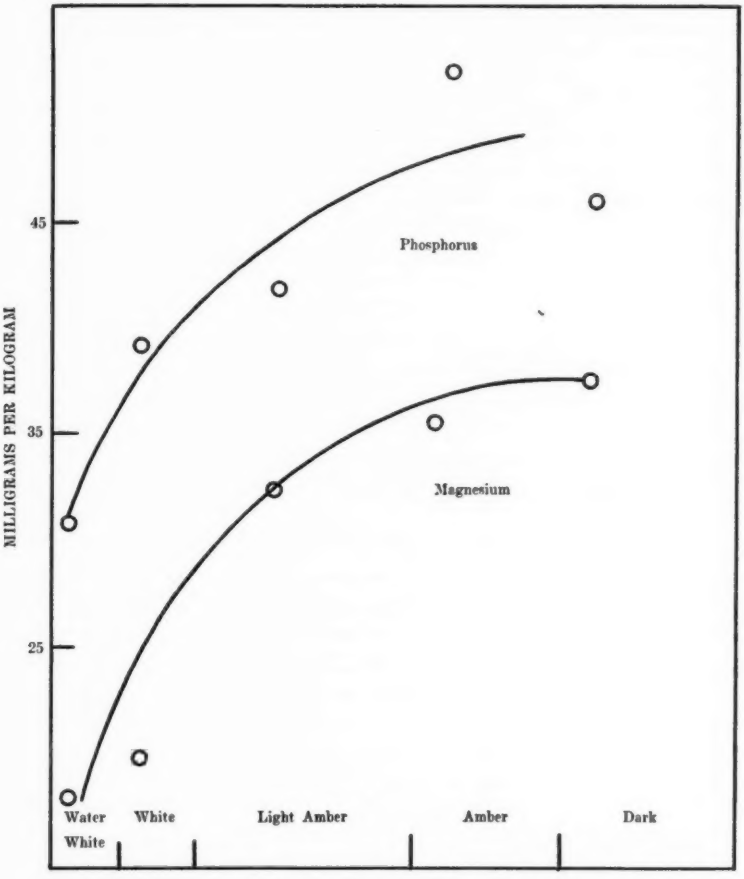

FIG. 1. Relation of phosphorus and magnesium content of honey to its degree of pigmentation.

grades but is not so marked in the case of phosphorus where there appears to be a downward trend in the deepest one, or dark. Similar treatment of the silica and calcium data would show an equally interesting parallelism. In this instance, both curves would show a slight

drop between the water-white and white color grades, a rise to a maximum in the amber, and a falling off in the most deeply pigmented group. When interpretation is given these curves in terms of predominating floral source of the samples which were examined throughout the course of this investigation, it appears that the buckwheat and wild-aster honeys of the North and the gallberry, tupelo gum, Mexican clover, and titi honeys of the South are poorer sources of calcium than either the white sage and orange-blossom honeys of California, the alfalfa honeys of the inter-mountain states, or the white-clover honeys of the Midwest. The situation is reversed, however, in so far as it affects phosphorus and, particularly, magnesium.

Comparison of the foregoing data on the three elements which comprise the subject of this communication with those reported by others presents some measure of difficulty because of lack of uniformity in the manner in which others have presented their findings. Some have seen fit to use the mg.-per-kg. or an equivalent method of expressing their data; others have calculated theirs on a per cent-of-ash basis; whereas a third group has used both methods. Reference to the findings of the first group, with reservations made in respect to all for doubtful accuracy because of failure to observe a probable source of error in the preliminary ashing of the sample, reveals the fact that the limiting values of phosphorus content (23 to 58 parts per million) which were found as a result of this study are in substantial agreement with those of Jewell (1931), who reported a 26-to-47 range for five Australian honeys, and with that of Van Dine and Thompson (1908), whose single report places a value of 34 parts upon an Hawaiian product, but not so with the summarized data pertinent to 112 honeys of European origin (Sweden, Switzerland, Austria, Hungary, Germany, British Isles, and Russia). In the latter instance it is only in the minimum value, which is 25, that any similarity is apparent, for here the maximum rises to 336 parts per million. It is Elser, on three occasions (1925-1928) presenting the literature with reports on the phosphorus content of some 20 honeys of Swiss and Austrian origins, respectively, who has consistently found higher values for this element; his maxima, in the order of geographical sources named, being four- and six-fold, those herein recorded. A similar analysis of the data which have been reported on a per cent-of-ash basis—they affect on the one hand some 200 honeys of European origins—reveals a range which lies between 1.30 and 35.5 per cent in contrast to the .84 to 9.55 variation (Table 2) noted herein. Nottbohm (1927) included in his studies five honeys which had been imported from South America and three which had been shipped to Germany from the United States. He found a fairly good parallel

in the variation of the phosphorus content of these honeys, of 6.77 to 8.80 per cent of ash in the first group and, similarly, 6.97 to 7.50 in the second. It is obvious that only in the order of magnitude of maximum values do these three groups of honeys from the western hemisphere show any similarity in phosphorus content.

In contrast to the relative abundance of information on the phosphorus content of honey, data extant on calcium are few; in the case of magnesium the paucity is even more marked. Information heretofore available as the result of the examination of 13 samples is to the effect that calcium will be found to comprise .47 to 6.90 per cent of the ash of honey. Although the lower limit will not be affected by this survey of more than 30 honeys, it appears that a higher maximum may be expected, or nearly double the former figure. The pertinent values are .46 to 13 per cent (Table 2). An extremely wide variation (41 to 670 parts per million) has been reported by those who have used the mg.-per-kg. form of expressing results. In these instances the number of samples—they form the subject of six reports—is the same as that examined here; the variation, likewise, is not confined to narrow limits for it is five to 266 parts per million.

As intimated above, magnesium as a constituent of honey seems to have aroused little interest, for in the two decades and more following the statement of Van Dine and Thompson (1908) that they had found 24 mg. per kg. of this element in an algaroba honey, no new information on this subject was contributed by others until Sundberg and Lundgren (1930) announced that the seven samples, all of Swedish origin, which they had analyzed contained 1.3 to 7.1 mg. per kg. and Jewell (1931) reported that the five Australian honeys which he had examined contained from four to 27 mg. per kg. In view of this situation, suggested extensions are perhaps more desirable than comparisons. To that end, therefore, it may be stated that a new upper limit of the magnesium content of honey has been found and that in the light of the examination of 47 honeys in which are included representative products from the United States, Panama, Sweden, and Australia, the concentration of this element in this food may be regarded as varying from one to 126 parts per million. When referred to the ash, the magnesium content will be found to lie between .66 and 11.47 per cent (Table 2), values which include in their compass the isolated data of Svoboda (1930 and 1933) who reported 2.61 and 3.70 per cent, respectively.

That phase of this investigation on honey minerals which centered around the alkalinity of the ash (Table 3), although admittedly too limited in its scope to justify a major deduction, indicates that alkaline values of honey probably closely parallel the ash content. Fur-

thermore, it appears to offer confirmation of a conclusion arrived at by Lothrop (1936), who used a different method of approach, that honey belongs to the alkaline-ash group of foods. The water-soluble constituents were found to predominate over the water-insoluble ones and this is true also of their respective alkalinities.

TABLE 3

Alkalinity of the Ash of Honey

Constituent	Predominant floral source		
	Orange blossoms	Buck-wheat	Titi
Ash [1]			
Total	0.047	0.159	0.263
Water-soluble	0.030	0.132	0.240
Water-insoluble	0.017	0.027	0.023
Alkalinity of ash [2]			
Water-soluble	3.6	16.2	34.5
Water-insoluble	2.5	4.7	5.1

[1] Per cent. [2] c.c. 0.1 *N* acid per 100 gm. of honey.

SUMMARY

In the examination of 35 samples deemed to include representatives of most of the honeys produced commercially in the United States, it has been found that there apparently exists a qualitative relationship between degree of pigmentation, as revealed by the present-day practice of color-grading this food, and mineral content. With the exception of the element calcium, the values found for the dark honeys were definitely greater than those noted in the case of the lighter-colored varieties in respect to silica, phosphorus, and magnesium. Comparison of the data obtained has been made with those reported by others for honeys produced, in the main, on foreign soil. No such high concentrations of either phosphorus (336 parts per million) or of calcium (670 parts), as revealed in a compilation of the reports available in the literature, were found. In this instance the respective limits proved to be 23 to 58 and 5 to 266 parts per million, in contrast to a 25-to-336 range for the former and the 41-to-670 variation for the latter. On the other hand, a new upper limit for the magnesium content of honey, of 126 parts per million, has been found, the heretofore existing maximum being 27 parts.

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EFFECTS OF CERTAIN METALS AND ALLOYS ON CLARET- AND SAUTERNE-TYPE WINES MADE FROM VINIFERA GRAPES

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(Received for publication, September 20, 1937)

The contamination of wines with metals and subsequent effects on the appearance, taste, and bouquet of the wines are of considerable importance in the wine industry. In this connection iron and copper have received considerable attention but there is comparatively little information concerning the effects of other metals on wines made from vinifera grapes.

The relation of iron to turbidity in wines has been discussed by Baragiola (1909, 1917), Fabre and Bremond (1927), Fonzes-Diacon (1917), Laborde (1917), Hugues (1929), Marcille (1931), Marsais (1930), Moreau (1917), Piedallu (1919, 1920), Seifert (1932), Trillat (1909), and Weil (1915). Byrne, Saywell, and Cruess (1937) determined the iron content of California grapes, musts, and wines and its loss from the musts during fermentation. Ribereau-Gayon (1929, 1930) studied the effect of copper as well as of iron on the clouding of wines. Carls (1918) and Benvegnin and Copt (1934) also considered the question of copper in wines. Ventre (1925) believed that manganese in wines produced a "casse," whereas according to Galletti (1923) small amounts of manganese improved wines. Braungard (1911) and Fillinger (1908) believed that wines were not affected by contact with aluminum, but Dubaquie (1925, 1926, 1930) found that aluminum and other metals dissolved from glass induced clouding of wine. According to Grelot (1922) aluminum, iron, tin, and zinc caused wines containing 25 mg. per liter of SO_2 to become turbid; but copper, lead, and nickel did not cause clouding when wines contained up to 450 mg. of SO_2 per liter. Searle, La Que, and Dohrow (1934) added various quantities of the citrate salts of aluminum, chromium, copper, iron, nickel, tin, and zinc to wines made from labrusca grapes grown in Canada and then observed them periodically for changes in taste and appearance. Small amounts of tin and iron caused turbidity, whereas even large quantities of nickel and zinc did not cause any perceptible changes. Copper and aluminum caused slight changes in appearance, and chromium tended to cause the

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wines to darken. They stated that changes in flavor and bouquet caused by metals are not very marked and that changes in color and brilliance of claret and white wines will be noted at much lower concentrations than changes of flavor and bouquet.

The writers have made a study of the effect of salts of several metals and the effect of certain metals and alloys on claret and sauterne wines made from vinifera varieties of grapes produced in California. Metallic ions were introduced into the wines quantitatively by adding known quantities of metal salts to 100-c.c. samples of wines which were then stored at room temperature until examined for changes in flavor, bouquet, and appearance. Standard solutions of

TABLE 1

Composition of Wines Used

	Sauterne type	Claret type
Alcohol	12.15%	12.90%
Volatile acid	.096%	.060%
Total acid	.465 grams per 100 c.c.	.660 grams per 100 c.c.
Reducing sugars	.103%	.223%
Tannin	.057 grams per 100 c.c.	.181 grams per 100 c.c.
Sulfur dioxide	128 parts per million	64 parts per million

metal salts were prepared in such a manner that when 10 c.c. of the solution was added to the wine sample it contained 2, 5, 10, 25, or 50 parts per million of metal. The wine samples containing the added salts and control samples containing added distilled water (10-c.c. aliquots) were stored in corked and paraffin-sealed eight-ounce bottles. Duplicate samples were examined visually and organoleptically by several individuals after 1, 7, 30, and 280 days of storage. The salts used were $Al_2(SO_4)_3$, Ag NO_3, Ca Cl_2 $CuCl \cdot 6H_2O$, $Fe_2(SO_4)_3 \cdot 9H_2O$, $Fe(SO_4) \cdot 7H_2O$, $K_2Cr_2(SO_4)_4 \cdot 12H_2O$, $NiCl \cdot 6H_2O$, $Pb(C_2H_3O_2) \cdot 3H_2O$, $SnCl_2$, $SnCl_4$, and $Zn(SO_4) \cdot 7H_2O$. A few tests were also made with $CdCl_2 \cdot 2H_2O$, Co $Cl_2 \cdot 6H_2O$, and Mn $Cl_2 \cdot 4H_2O$.

The qualitative tests were made by introducing 15 grams of lathe turnings into a pint bottle containing 400 c.c. of wine. The bottles were corked, shaken, and then stored at room temperature for several weeks. Frequent observations were made for changes in bouquet and appearance. The metal and alloy turnings added were Allegheny C, aluminum, aluminum-alloys 12 and 76, aluminum-bronze, acid-bronze, cast iron, cupro-nickel, copper, Durimetl, Duriron, Everdur, machine-brass, manganese-bronze, Monel metal, phosphorous-bronze, steel, tin, and Waukesha-alloy. The composition of most of these metals and alloys has been given by Mrak, Caudron, and Cash (1937).

The wines used in all tests were clear and free of sediment at the time the test samples were prepared. The control samples remained clear in all instances, although traces of sediment were observed in some of the samples stored for a long period of time. Compositions of the wines used are given (Table 1).

RESULTS OF QUANTITATIVE TESTS

The observations on wines to which metallic salts were added show that the effect of metals on appearance, bouquet, and taste of the wines varied with the length of storage, concentration of metal in the wine, and type of wine (Tables 2 and 3). In most instances the concentration of metal causing detectable changes decreased when the length of storage period was increased. In certain samples changes occurred after short periods of storage, which disappeared upon continued storage.

Effect of Metals on Appearance of Wines: All metals except nickel, chromium, zinc, and stannous tin in claret and sauterne wines, and ferrous iron in claret wine, caused turbidity. The turbidity of white wine caused by aluminum, silver, ferrous iron, or ferric iron precipitated out upon continued storage. The turbidity in red wine caused by aluminum or stannic tin acted in a similar manner. As little as one or two parts per million of aluminum, copper, lead, or stannic tin were sufficient to produce turbidity in white wine. This turbidity, resulting from the presence of copper, lead, or tin, formed slowly but was easily observed after 280 days of storage. Claret wine became turbid when it contained one or two parts per million of ferric iron, lead, or copper and slightly higher concentrations of calcium or silver; but it was not very sensitive to aluminum or tin. Turbidity in claret wine caused by calcium, copper, ferric iron, lead, and silver was present after the samples had been stored 280 days. Cadmium, cobalt, or manganese did not cause noticeable changes in appearance in either sauterne or claret wine during three weeks of storage.

Chromium, lead, silver, stannous tin, and zinc caused sauterne wine to darken; whereas iron, nickel, and calcium caused claret wine to darken. The discoloration caused by iron was very distinct but that caused by calcium or nickel was indistinct.

Effect of Metals on Bouquet of Wines: The data show that in most instances changes in bouquet occurred very slowly and were not observed until after a considerable period of storage (Tables 2 and 3). All metals added as metallic salts except calcium, copper, ferrous iron, or stannous tin in white wine or aluminum, copper, or ferrous iron in red wine caused the formation of a definite aldehyde odor. This odor was more intense and objectionable in the samples

TABLE 2

Minimum Concentration of Metal Causing Detectable Changes in Taste, Appearance, and Bouquet of White Wine After Various Storage Periods

(Given in parts per million)

Storage period in days	Appearance				Bouquet				Taste			
	1	7	30	280	1	7	30	280	1	7	30	280
Aluminum	25 T[1]	2 T	....	2 S	N	N	....	1 A	N	25	....	2
Calcium	N	N	N	25 T	N	N	N	2 F	N	N	N	2
Chromium	N	N	10 D	2 D	N	N	N	1 A	N	10	10	1
Copper (cupric)	N	10 T	10 T	2 T	N	N	N	N	2	2	2	2
Iron (ferrous)	10 T	10 T	10 S	10 S	N	N	N	N	N	10	10	2
Iron (ferric)	N	N	25 T	2 S	N	N	N	10 A	N	N	N	2
Lead	N	N	10 T	2 D	N	N	N	10 A	N	N	25	10
Nickel	N	N	N	N	N	N	N	2 A	50	10	10	1
Silver	25 T	2 S	2 SD	2 SD	N	N	25 A	10 A	25	10	10	10
Tin (stannous)	N	N	N	5 D	N	N	N	1 F	25	25	25	5
Tin (stannic)	N	N	25 T	1 T	N	N	N	1 A	N	25	25	1
Zinc	N	50 S	5 D	5 D	N	N	N	5 A	N	25	25	5

[1] A=aldehyde odor; D=darkened; F=flat, bouquet lacking; N=no noticeable change with 50 p.p.m. of metal; S=sediment; T=turbid.

TABLE 3

Minimum Concentration of Metal Causing Detectable Changes in Taste, Appearance, and Bouquet of Red Wine After Various Storage Periods

(Given in parts per million)

Storage period in days	Appearance				Bouquet				Taste			
	1	7	30	280	1	7	30	280	1	7	30	280
Aluminum	25 T[1]	25 T	....	50 S	N	N	....	N	N	50	....	5
Calcium	N	N	N	5 TD	N	N	N	5 A	N	N	N	5
Chromium	N	N	N	N	N	N	N	2 A	N	25	25	2
Copper (cupric)	N	10 S 50 T	10 S 50 T	2 T	N	N	N	N	5	5	5	5
Iron (ferrous)	N	N	50 S	10 S	N	50 F	50 F	50 F	25	25	10	5
Iron (ferric)	50 T	50 T	50 T	1 TD	N	N	N	10 A	N	N	N	1
Lead	N	N	10 D	1 TS	N	N	5 A	1 A	N	N	50	1
Nickel	N	N	25 S	1 DS	N	N	N	5 A	25	10	10	5
Silver	50 T	50 T	10 T	10 T	N	N	25 A	10 A	25	25	25	5
Tin (stannous)	N	N	N	5 S	N	N	N	2 A	25	25	25	2
Tin (stannic)	N	50 T	25 T	25 S	N	N	N	1 A	50	25	25	1
Zinc	N	N	N	N	N	N	N	10 A	25	25	25	10

[1] A=aldehyde odor; D=darkened; F=flat, bouquet lacking; N=no noticeable change with 50 p.p.m. of metal; S=sediment; T=turbid.

containing the higher concentrations of metal. Trillat (1909) reported the rapid formation of aldehyde by wines that had been in contact with iron. He found the chemical composition of the wine to be a very important factor affecting this type of aldehyde formation. This may explain the failure of Searle, La Que, and Dohrow to observe aldehyde formation in the wines they tested.

Although certain metals did not induce the formation of noticeable quantities of aldehyde, they destroyed the original bouquet of the wine. In the tables these have been designated as flat wines. Only three metals—ferrous iron in white wine, aluminum in red wine, and copper in either wine—failed to cause detectable changes in bouquet.

Effect of Metals on Taste of Wines: All the metals used caused changes in taste providing the samples were stored for a sufficient period of time. Relatively low concentrations of metals produced distinct changes in taste in the samples stored for 280 days. The white wine was most sensitive to stannic tin, nickel, and chromium; whereas the red wine was most sensitive to stannic tin, lead, and ferric iron. Cadmium, cobalt, and manganese did not cause any noticeable changes in taste after three weeks of storage but this does not prove that changes would not have occurred after several months of storage.

RESULTS OF QUALITATIVE TESTS

These tests were conducted to determine the effect of various metals and alloys in the form of lathe turnings on the appearance and bouquet of claret and sauterne wines. The test period was for four weeks. The conditions existing in these tests would probably never be duplicated in a winery, but it is believed the great surface of exposed metals accelerated changes that might occur in a winery under less severe conditions. Duriron, Durimetl, Inconel, Allegheny C, aluminum-bronze, Monel metal, and cleaned copper did not cause any noticeable changes in appearance or bouquet of the wines after four weeks of storage in the presence of these metals (Table 4). Uncleaned copper, on the other hand, caused a very definite change in the appearance of the wines.

Hydrogen sulfide formed when some of the less resistant metals, such as cast iron, steel, tin, and some of the aluminum-alloys, were added to the wines. Aluminum and manganese-bronze definitely bleached claret wine. The changes caused by cupro-nickel, Waukesha-alloy, machine-brass, phosphorous-bronze, Everdur, and acid-bronze were very slight and not easily detected.

SUMMARY AND CONCLUSIONS

1. Experiments were conducted to determine the effect of metals on the appearance, bouquet, and taste of claret- and sauterne-type wines made from vinifera grapes.

2. The effect of metals on wine varied with the type of wine, metal, metal concentration, and length of storage period.

3. The concentration of dissolved metal required to cause detectable changes in appearance, bouquet, or taste decreased as the time of storage of the wine was increased.

TABLE 4

Effect of Metal Turnings on Appearance and Bouquet of Wines

Metal	Sauterne wine		Claret wine	
	Appearance	Bouquet	Appearance	Bouquet
Aluminum pure	T[1]	N	Pale	N
Aluminum 76	V.T.	H_2S	V.T.	H_2S
Aluminum 12	V.T.	H_2S	....	....
Aluminum-bronze	N	N	N	N
Acid-bronze	N	N	Sl.T.	N
Everdur	N	N	Sl.T.	N
Manganese-bronze	Sl.T.	....	Pale	N
Phosphorous-bronze	Sl.T.	N	N	N
Machine-brass	Sl.T.	N	N	N
Waukesha-alloy	Sl.T.	N	Sl.T.	N
Cupro-nickel	Sl.T.	N	Sl.T.	N
Monel metal	N	N	N	N
Copper (clean)	N	N	N	N
Copper (dirty)	T	N	V.T.	N
Tin	Green D.T.	N	V.T.	H_2S
Cast iron	V.T.	H_2S	T	H_2S
Steel	T	H_2S	V.T.	H_2S
Durimetl	N	N	N	N
Duriron	N	N	N	N
Inconel	N	N	N	N
Allegheny C	N	N	N	N

[1] D=darkened; N=no change; Sl=slightly; T=turbid; V=very.

4. All metals, added to wine as salts, except nickel, chromium, zinc, or stannous tin in claret and sauterne wine or ferrous iron in claret wine caused the wines to become turbid. In certain instances turbidity disappeared upon prolonged storage.

5. Changes in bouquet occurred slowly in most instances. All metals except calcium, ferrous iron, and tin in white wine or aluminum, copper, or ferrous iron in red wine caused the wines to have a definite aldehyde odor providing the samples were stored for a sufficient period of time.

6. All metals added to the wines as salts caused changes in taste, providing the samples were stored for a sufficient period of time.

7. Turnings of all of the metals and alloys used except Duriron, Durimetl, Inconel, Allegheny C, aluminum-bronze, and Monel metal caused changes in appearance or bouquet. When copper was thoroughly cleaned before grinding and adding to the wines, it had no detectable effect on appearance; but when turnings made of uncleaned copper was added to the wines, turbidity developed rapidly.

ACKNOWLEDGMENT

The writers wish to express their gratitude to Dr. W. V. Cruess and Dr. M. A. Joslyn for the assistance and coöperation given during the course of this investigation.

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LOSSES OF VITAMIN C DURING COOKING OF NORTHERN SPY APPLES[1]

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Comparatively few studies have been made concerning the occurrence or stability of vitamin C in apples, or the influence of various factors, such as variety, storage, temperature, age of tree, etc., on the vitamin-C content of this economically important fruit. The meager research on the apple may be attributed to the reports of early workers that it was not a good source of vitamin C.

A wide variation in the vitamin-C content of different varieties of apples has been reported by several investigators. Smith and Fellers (1934) made an extensive survey of previously reported data and determined the potency of 21 varieties of Massachusetts-grown apples by biological assay. They found that the daily protective level for guinea pigs ranged from four grams for Baldwin apples to over 25 grams for McIntosh apples.[2]

Several investigators, including Bracewell and coworkers (1931), reported that the epidermis of the apple was a more active source of vitamin C than the cortex. Bracewell found the skin to be from three to four times more active than the whole apple. However, in a more recent publication Manville and McMines (1936) pointed out that careful removal of the peel did not markedly decrease the vitamin-C content.

The loss of vitamin C during the cooking of apples was investigated by Kohman, Eddy, and Carlsson (1924) who found that practically all the vitamin C was lost in freshly prepared apple sauce, baked apples, or apples canned without special treatment. Hessler

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 217, August 31, 1937. Publication No. 351 from the Chemistry Department, University of Pittsburgh. Presented in part before the Division of Biological Chemistry at the 94th meeting of the American Chemical Society on September 7, 1937. This paper is an abstract of a thesis presented to Cornell University by Katherine M. Curran in partial fulfillment of the requirements for the degree of Master of Science.

[2] Recalculated on the basis that .5 mg. of ascorbic acid per day is required for the protection of a guinea pig from scurvy [Waugh and King (1932)], the Baldwin apple contained .125 mg. per gm. of vitamin C and McIntosh .02 mg. per gm.

and Williams (1929) reported considerable loss of the vitamin in fresh apple sauce cooked in an open kettle. These results were confirmed by Fellers and coworkers (1933). In addition, they found that canned Baldwin sauce prepared from peeled, cored fruit was somewhat richer than the strained sauce made from the unpeeled fruit.

The purpose of this investigation was to study further the effects of different methods of cooking apples in relation to vitamin-C losses.

EXPERIMENTAL PROCEDURE

Analytical Methods: The Northern Spy variety of apple was used since it was found to be a good source of vitamin C and yielded a light-colored extract which could be titrated easily. The apples used in this investigation were obtained from the State College of Agriculture, Cornell University. They were harvested during the last week of October and placed in storage at 0 to 2°C. (32 to 35.6°F.).

The method used in determining the ascorbic acid content of the apples was essentially that of Bessey and King (1933) as modified by Mack and Tressler (1937). In order to be certain that all of the biologically active ascorbic acid was measured, an aliquot of the sulphuric acid extract was treated with hydrogen sulphide for a period of 10 minutes and allowed to stand in a corked test tube for 20 minutes. Carbon dioxide was then bubbled through the solution until a negative test for hydrogen sulphide was obtained. Thirty milliliters of eight-per cent acetic acid were added to the reduced solution and it was titrated immediately. This procedure is a modification of that proposed by Tillmans, Hirsch, and Jackisch (1932). Throughout the entire experimental period there appeared a slight but regular increase in titratable ascorbic acid after reduction with hydrogen sulphide.

Vitamin-C determinations were calculated on both a wet-weight and a dry-weight basis. The raw apples were sampled by taking 20-gram radial sectors from both the peeled and unpeeled apple from which the core had been removed. This gave a fairly representative sample of skin and flesh. Duplicate samples were taken from opposite sides of the apple. In the peeled apple the skin was removed as thinly as possible in order to reduce the loss of the vitamin.

Determinations were also made on raw, quartered samples which had been allowed to stand at room temperature for one-, two-, and three-hour periods.

For purposes of biological assay, samples of raw, unpeeled apples and strained apple sauce were frozen and shipped in a container with dry ice to the University of Pittsburgh. The curative type of

assay, previously described by Tressler, Mack, and King (1936), was used as a measure of the ascorbic acid potency.

The assay was partially completed before it became apparent that during freezing of the raw, unpeeled apples, there was a considerable loss of vitamin C. Upon redetermination the weighed portions of apple which were being fed to the test animals to represent .5 mg. of ascorbic acid were found to contain only .33 mg. or .076 mg. of ascorbic acid per gram (indophenol titration value after reduction with hydrogen sulphide). The feeding level of .33 mg. was continued for the remainder of the assay. For comparison other groups received .5 mg. and 1.0 mg. of pure vitamin per day fed from graduated pipettes. The negative control animals continued to receive the basal diet only.

Cooking Procedures: a. Unstrained apple sauce. Peeled, quartered, and cored apples were cooked in a covered three-quart enameled pan with an inside diameter of seven inches. The peeled apples (480 gm.) were dropped into 225 ml. of rapidly boiling water and cooked until tender (18 min.). At the done stage, previously determined by the common household method of testing with a fork, 60 gm. of granulated sugar were added. The gas inflow was regulated so that the rate of cooking and evaporation were uniform and there was little difference in the weight of the apple sauce obtained from each cooking.

During the preliminary experimental work, it was found that exposure of the hot sauce to the air for the short time required for stirring and sampling resulted in a considerable variation in its vitamin-C content. In order to obtain more uniform results and to prevent further oxidation, stirring and sampling were done under an atmosphere of carbon dioxide. Samples of approximately 20 gm. were removed from the hot sauce and placed in chilled, covered weighing bottles containing 35 ml. of extracting acid, cooled rapidly to room temperature, weighed, and analyzed.

In order to determine the period of greatest loss of vitamin C during cooking, unstrained sauce was made with 60 gm. of sugar added at the beginning of the cooking period. Samples were removed for analysis at the end of 4, 8, 12, and 18 minutes.

b. Strained sauce. Four hundred and eighty grams of unpeeled apples were quartered, cored, and cooked under the same conditions as the unstrained sauce. At the end of the cooking period, the sauce was allowed to stand (covered) until it had cooled to room temperature (1⅓ to 1½ hours). The pulp was then forced through a wire sieve, and thoroughly mixed with 60 gm. of sugar. The sauce was weighed and samples removed for titrating as in the unstrained sauce.

c. Baked apples. Apples of approximately the same size (3 to 3½ inches in diameter) and weight (6 ounces) were rinsed, cored, scored, and baked in a pyrex casserole for 1 to 1⅓ hours at 204°C. (400°F.). At each baking 60 ml. of water were put in the bottom of the dish and 12 gm. of sugar placed in the cored center of each apple.

TABLE 1

Biological Assays of Ascorbic Acid in Northern Spy Apples Grown in Tompkins County, New York

Test food	Wt. of apple fed	Vitamin C fed per day	Number of animals	Av. wt. at beginning of test	Av. wt. gain in 21 days	Scurvy score
	gm.	*mg.*				
Apple, raw, unpeeled	4.3	0.33	8	317	— 4	4.5
Apple sauce, strained	7.1	0.5	8	324	+ 22	3.4
Ascorbic acid solution	.....	1.0	5	348	+ 76	1.0
Ascorbic acid solution	.....	0.5	9	328	+ 12	3.9
Basal diet	.....	0.0	5	350	—132	15.0

After baking, radial sectors were removed from each apple and placed in a covered weighing bottle containing 35 ml. of extracting acid. The samples were rapidly chilled and analyzed. Determinations were made on apples baked in both covered and uncovered casseroles.

d. Apple pie. Five hundred grams of freshly peeled, cored apples were used for each pie. Weighed portions of pastry, sugar, and

TABLE 2

Ascorbic Acid Content of Raw Apple and Apple Sauce

Exp. No.	Raw apple (mg. per gm.)				Apple sauce, cooked 18 min. (mg. per gm.)			
	Unpeeled		Peeled		Unstrained		Strained	
	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.
1	.12	.76	.11	.72	.07	.37	.06	.35
2	.11	.76	.10	.72	.07	.34	.07	.41
3	.10	.73	.09	.67	.07	.31	.07	.41
4	.12	.79	.10	.70	.06	.33	.07	.46
Av.	.11	.76	.10	.70	.07	.34	.07	.41

butter were used so as to keep the proportion of ingredients in all pies uniform. The pies were baked in nine-inch granite-ware pie plates at 218°C.(425°F.) for 20 minutes and at 190°C.(375°F.) for the remaining 40 minutes of the baking period. All pies were weighed before and after baking. In computing the vitamin losses the change in the weight of the pastry during baking was taken into consideration. Determinations were made on apples from freshly baked pies

and from pies which had remained at room temperature for 3, 12, 24, and 48 hours respectively.

DISCUSSION

The results of the biological assay (Table 1) are in good agreement with the titration values. The growth response and scurvy scores for animals receiving the test food agreed well with those receiving standard solutions of vitamin C.

TABLE 3

Ascorbic Acid Losses in Raw Quartered Samples of Apple on Standing at Room Temperature

Time in hours	Ascorbic acid		Loss	
	Peeled	Unpeeled	Peeled	Unpeeled
	mg. per gm.	*mg. per gm.*	*pct.*	*pct.*
0	.10	.11	0	0
1	.08	.09	20	19
2	.08	.08	20	20
3	.07	.07	30	36

On a percentage basis, peeling caused a loss of from eight to 17 per cent of the ascorbic acid in the apples (Table 2) as compared with an equal weight of unpeeled fruit.

Approximately one-fifth of the ascorbic acid contained in raw, quartered samples of peeled and unpeeled fruit was lost on standing

TABLE 4

Ascorbic Acid Content of Apples During Cooking of Unstrained Sauce

Cooking period	Ascorbic acid	Original ascorbic acid [1]
min.	*mg. per gm.*	*pct.*
0 (raw)	.100	100
4	.070	76
8	.064	69
12	.063	68
16	.065	70
18 (done)	.060	67

[1] These figures represent percentage of the total ascorbic acid remaining in the sauce.

at room temperature for one- and two-hour periods. At the end of three hours the loss was increased to about one-third of the total original ascorbic acid present in the fresh fruit (Table 3).

Both the unstrained and strained apple sauce (Table 2) contained .07 mg. per gm. The percentage of vitamin C retained in one cooking

of apple sauce, however, was slightly higher (75 per cent) in the unstrained sauce than in the strained sauce (68 per cent). The total weight of each cooking of the unstrained sauce averaged about 530 gm., while that of the strained sauce averaged 518 gm. This greater loss may be explained by waste in straining or further oxidation during straining.

TABLE 5

Ascorbic Acid Content of Apples Baked in Covered and Uncovered Baking Dishes

		Experiment No. 1	Experiment No. 2	Experiment No. 3	Experiment No. 4	Apparent loss
		mg. per gm.	*mg. per gm.*	*mg. per gm.*	*mg. per gm.*	*pct.*
Uncovered baking dish	Wet wt.	.033	.032	.031	.032	....
	Dry wt.	.10	.13	.13	.12	80
Covered baking dish	Wet wt.	.026	.034	.031	.031	....
	Dry wt.	.11	.16	.16	.14	81

The greatest loss of vitamin C in the unstrained sauce occurred during the first four minutes of cooking (Table 4, Fig. 1). At the end of that period there was a loss of 23 per cent of the vitamin Fig. 2). The loss during the remainder of the cooking period was negligible.

There was practically no difference in the loss of vitamin C during the baking of apples in covered or uncovered casseroles. The loss

TABLE 6

Ascorbic Acid Content and Percentage Loss in Apple Pie After Baking and Holding

(Average of four experiments)

Time in hours	Ascorbic acid		
	Wet wt.	Dry wt.	Apparent loss [1]
	mg. per gm.	*mg. per gm.*	*pct.*
Before baking	.100	.700	0
0	.022	.090	80
3	.024	.100	78
12	.023	.100	79
24	.022	.100	82
48	.013	.053	88

[1] These figures represent the percentage loss of the total ascorbic acid remaining in the apple filling of the pies.

(Table 5) was from 80 to 81 per cent. Apparent factors influencing the loss of ascorbic acid were the length of time in baking, the relatively slow rate of heat penetration with a corresponding delayed effect on the oxidation enzyme, and the presence of atmospheric oxygen.

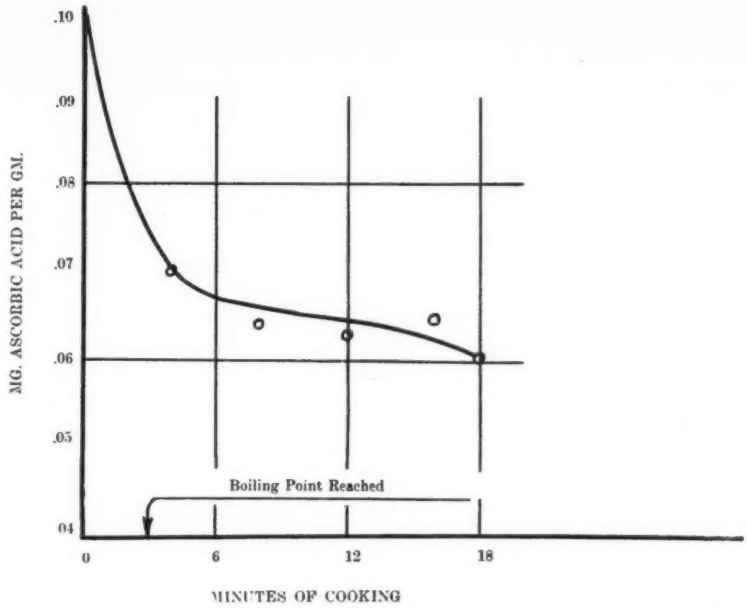

FIG. 1. Loss of ascorbic acid during cooking of Northern Spy apples for sauce.

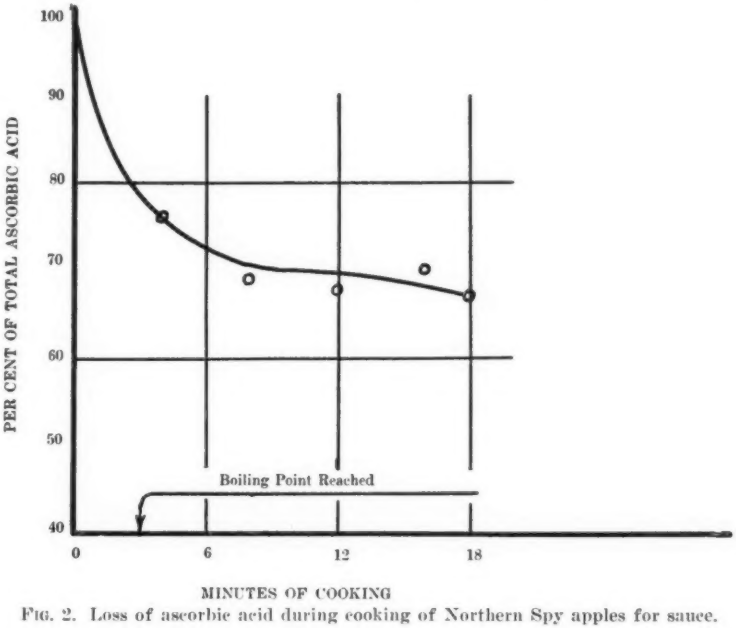

FIG. 2. Loss of ascorbic acid during cooking of Northern Spy apples for sauce.

These results are in agreement with Kohman (1924) but contrary to the findings of Bracewell and coworkers (1930), who reported little destruction of vitamin C during baking of Bramleys Seedlings. The method of baking used may account for the slight loss reported by Bracewell, who placed apples in a drying oven previously heated to 160°C.(320°F.) and held them at that temperature for 50 minutes or until the apples were soft.

There was a loss of approximately 80 per cent of vitamin C during the baking of apples in pie. On standing at room temperature a gradual loss continued until, at the end of 48 hours, the total loss was about 88 per cent of the original value (Table 6).

CONCLUSIONS

1. The vitamin-C content of raw Northern Spy apples grown in Tompkins County, New York, was found to be about .11 mg. per gm.

2. Apple sauce made from peeled and unpeeled apples contained .07 mg. per gm. of vitamin C. The unstrained sauce made from peeled apples showed a slightly greater retention (75 per cent) of the total original ascorbic acid than the strained sauce made from unpeeled fruit (68 per cent).

3. In the case of apple sauce made from peeled apples the greatest loss of vitamin C took place during the first four minutes of cooking.

4. There was a loss of approximately 80 per cent of the vitamin C during the baking of apples.

5. During the baking of apple pie, 80 per cent of the original vitamin C was lost. After the pie had stood for 48 hours, this loss was increased to 88 per cent.

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EFFECTIVENESS OF COTTON-SWAB METHODS IN BACTERIOLOGICAL EXAMINATION OF PAPER ICE CREAM CONTAINERS[1]

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(Received for publication, September 20, 1937)

Much attention has been directed recently to the sanitation of utensils, glassware, and plateware in public eating places since these have, in some instances, transmitted pathogenic bacteria. Bacteriological standards and methods for examination of eating utensils have been suggested and, in some cases, already adopted by health departments. As an alternative single-service utensils and containers ordinarily of paper are usually permitted, apparently on the assumption that they are sanitary, although few bacteriological investigations of paper containers have been reported. Owing to the lack of standard procedures for sampling such containers, the efficiency of usual procedures for sampling eating and drinking utensils and other methods were compared.

Since paper ice cream containers are used in retail food stores, confectioneries, restaurants, and similar establishments that may be operating under bacteriological regulations for containers and utensils which are re-used, the methods of sampling and standards adopted for re-used containers may be considered. Fellers and Levine (1936) investigated glassware for dispensing beverages and proposed a standard method of sampling, using sterile cotton swabs in three c.c. of .8-per cent sodium chloride in ordinary 25-c.c. culture tubes. The moistened swab was used to swab the utensil thoroughly and was then replaced in the tube and returned to the laboratory. The tube was agitated by holding it firmly in the hand and striking 25 times; the swab was removed, pressed against the inside of the tube to remove excess liquid, and the entire liquid contents poured into a petri dish and mixed with nutrient agar. Fellers also compared 18 different methods of sampling glasses and dishes for utility, accuracy, speed, and simplicity. The swab methods always detected over 40 and often 75 per cent of organisms on glasses artificially inoculated with pure cultures, although with mixed cultures recovery was always less. The

[1] Based on a portion of a thesis submitted to the Graduate School of the University of Maryland by the senior author in partial fulfillment of the requirements for the degree of Master of Science.

agar-disc method was found too difficult to use and gave only 50 per cent recovery of organisms artificially inoculated on glasses, and other methods were found inaccurate and impractical.

The District of Columbia Health Department (Feb. 28, 1936) adopted a procedure essentially the same as that later proposed by Fellers. The subcommittee on standard methods for the examination of dish-washing devices of the American Public Health Association (1936-1937) proposed a standard procedure for the bacteriological examination of plateware. In this test a single damp swab was passed over a four-square-inch area of each of 10 plates, with the swab agitated between swabbings in 10 c.c. of sterile salt solution. The suspension was plated as usual and the number of organisms per surface area was determined.

In studying the effectiveness of dish and utensil washing Krog and Dougherty (1936) wiped a moist sterile swab three times around the portion of glass coming in contact with the user's lips and then placed the swab in a tube containing five c.c. of sterile water. The swab handle was adjusted in the plug so the lower half of the swab was submerged, the tube shaken violently against the palm of the hand 50 times, the swab then pressed out against the inside of the tube, and one c.c. of the suspension plated out. Similar procedures have been used by the Charlotte Department of Health, New York City Department of Health and others, as reported by the Public Health Committee of the Cup and Container Institute.

There have been no reports on numbers of organisms retained in cotton swabs after wiping over a surface followed by the usual methods of elution. However, Dearstyne (1918) showed definitely that strands of cotton from plugs used in dilution bottles retained numerous bacteria. The exudate from the cotton plug always contained less bacteria than fluid shaken against it, and if the plug was allowed to drain dry or was compressed to lose all moisture, about 20 organisms per 100 were still retained.

EXPERIMENTAL PROCEDURE

Swabs used in this work consisted of cotton wound on the end of a six and one-half inch wooden applicator to a thickness of one-quarter inch and breadth of one inch. The swabs were wrapped in pairs in brown paper and sterilized by autoclaving at 120°C.(248°F.) for 30 minutes, as were the dilution blanks containing 40 c.c. of distilled water. Standard nutrient agar adjusted to pH 7.0 was used for plating. Plates were poured in duplicate and incubated at 37°C. (98.6°F.) for 48 hours.

The first experiments were to determine the efficiency and advisability of employing usual methods of sampling with cotton swabs. The inside surface of the paper container was thoroughly wiped out with a damp swab which was then agitated in the dilution blank and finally pressed out against the shoulder and neck of the dilution bottle. Next, this same swab was placed in a second dilution blank by breaking off the portion of wooden applicator with the cotton

TABLE 1

Inefficiency of Using One Swab and Only Pressing It Out in Determining Number of Bacteria in Open-Top Paper Containers

Container	Location in nest	Total bacteria removed	Percentage of total organisms recovered by		
			Pressing out	Shaking out	
			Moist swab 1	Moist swab 1	Dry swab 2
1	Top	5,180	63.3	17.0	19.7
2	2nd	80	100.0	0.0	0.0
3	3rd	500	76.0	12.0	12.0
4	Top	13,380	80.3	2.4	17.3
5	2nd	1,600	63.7	18.7	17.6
6	3rd	480	62.5	12.5	25.0
7	4th	2,340	29.9	63.2	6.9
8	Top	600	10.0	46.6	43.4
9	2nd	840	64.3	28.6	7.1
10	3rd	740	43.2	48.6	8.2
11[1]	Top	280	21.4	28.5	50.1
12[1]	2nd	160	100.0	0.0	0.0
13[1]	3rd	80	100.0	0.0	0.0
14	Top	4,900	71.8	13.8	14.4
15	2nd	7,160	47.5	13.7	41.8
16	3rd	360	22.4	0.0	77.8
17	Top	10,580	52.3	44.0	23.7
18	2nd	2,960	74.3	17.0	8.7
19	3rd	2,060	22.3	48.5	29.2
20	Not top	480	70.7	12.7	16.6
Average percentage recovery..	.....	.....	58.0	21.0	21.0

[1] Pint size; all others quart.

swab directly into the blank, being careful that none of the portion in the blank had been touched by hand. Then, using a second, but *dry* swab, the container was again wiped out and the swab aseptically broken into a third dilution blank as above. These dilution blanks were shaken until the swabs were well disentangled, and one-c.c. aliquots plated in duplicate.

The inefficiency of using just one swab and only pressing it out, can be seen from results (Table 1). Of the total organisms found in the container, an average of only 58 per cent was recovered by

pressing out the first swab in the dilution blank. By shaking out this same swab, additional organisms were found, averaging 21 per cent of the total organisms demonstrated; and swabbing with a second swab yielded an additional 21 per cent. It is not presumed that these two swabs removed all organisms in the container; however, by shaking out these two swabs probably most organisms were recovered and a truer picture of the bacteriological condition of the container obtained.

In a further search for a suitable method for sampling paper ice cream containers a modification of the agar-disc method was tested. This consisted of pouring agar in the box to be sampled, the center portion of which was taken up by a sterilized box of smaller size in order to save media, and the top of the agar between the two boxes protected from outside contamination by cotton. Since this was found to be impractical and unsatisfactory, its use was discontinued.

A rinse method used on cylindrical containers with close-fitting tops was satisfactory for this type of container. However, such a method for sampling open-top rectangular containers seemed unreasonable, since it would be impossible to rinse thoroughly their entire inner surface without some diluent escaping from the loosely closed top. Accordingly the following procedure embodying both the moist and dry swabs previously used was selected and employed throughout the remainder of the investigation. Two swabs held in the same hand, the first moistened from the 40-c.c. water blank used and the second dry, were used to swab the inside of the container thoroughly. Both swabs were broken into the 40-c.c. dilution blank and shaken until disentangled, when one-c.c. aliquots were plated in duplicate. The average counts were reported as bacteria per container.

To again establish the efficiency of the method employed, a second series of experiments was carried out, using sterilized paper containers artificially inoculated with mixed cultures. Quart sizes of open-top rectangular ice-cream boxes were placed in brown paper bags and sterilized by autoclaving. Eighteen organisms isolated in this work were grown on agar slants for 18 to 24 hours, washed off with two c.c. of sterile saline and .1 c.c. of each suspension pooled. The pooled suspension was adjusted, using a nephelometer, diluted $\frac{1}{40}$ with sterile saline and used to inoculate the sterilized container by means of a sterilized nasal atomizer. Some suspension was placed in the glass chamber of the atomizer, the top screwed on, the entire atomizer placed on a level surface, and the suspension sprayed into the air until the liquid fell below the atomizer tube originally submerged and the spray ceased. Then an additional .1 c.c. of suspension was

placed in the chamber, the top tightened to the same position as before, and this increase in volume was sprayed directly into the sterilized container. The amount of inoculation was controlled automatically since the spray again ceased as soon as the .1 c.c. of added volume was exhausted. The accuracy of this procedure was determined gravimetrically, the amounts added and sprayed out checking satisfactorily.

The inoculated containers were allowed to stand about one hour, which afforded sufficient time for moisture introduced to evaporate

TABLE 2

Efficiency of Swabbing Out Inoculated Containers With Wet and Dry Swabs

Container	Total bacteria removed	Percentage of total organisms recovered by			
		1 wet and 1 dry swab	3rd swab (moist)	4th swab (moist)	5th swab (moist)
1	3,440	84.9	8.1	7.0	6.5
2	3,060	81.0	9.8	9.2	6.3
3	2,980	87.2	8.0	4.8	1.4
4	4,180	83.2	9.0	7.8	3.4
5	4,620	93.5	3.0	3.5	4.2
6	2,320	81.9	8.1	10.0	.....
7	3,540	80.8	13.5	5.7	.....
8	2,200	85.5	9.1	5.4	.....
9	1,900	95.8	3.1	1.1	.....
10	2,840	80.3	13.4	6.3	.....
11	1,160	89.7	6.9	3.4	.....
12	780	71.8	17.9	10.3	.....
13	1,860	86.0	6.4	7.6	.....
14	1,200	93.3	5.0	1.7	.....
15	10,760	76.6	14.1	9.3	.....
16	3,280	84.1	6.1	9.8	.....
17	4,760	53.8	20.2	26.0	.....
18	4,760	63.8	21.0	15.2	.....
19	3,280	80.5	14.6	4.9	.....
20	3,580	90.5	5.6	3.9	.....
Average percentage recovery	.....	83.0	10.0	7.0	.....

or be absorbed. They were then wiped out with one wet and one dry swab, simultaneously, having the dry swab immediately following the wet one. This was followed by two separate swabbings, each with a single wet swab, and in five samples by a third additional wet swab. These swabs were placed in their respective dilution blanks, well shaken, and duplicate plates poured from each.

Results indicating the efficiency of the method used are given in percentages calculated from the total number of organisms recovered from the artificially inoculated box by four swabs (Table 2). An

additional swabbing in five instances revealed that some organisms still adhered to the container, a number considerably less than recovered by the previous swab, however. On the basis that 100 per cent was recovered by four swabs, an average of 83 per cent was recovered by using one wet and one dry swab simultaneously, and an average of 10 per cent with the first and seven per cent with the second additional swab.

DISCUSSION

One swabbing of the paper container with a wet and a dry swab, simultaneously, followed by thorough disentangling in the dilution blank appeared to be satisfactory, since this gave an average recovery from inoculated containers of 83 per cent of organisms actually obtained when using four swabs. Although recovery by this method varied from 53 to 95 per cent, it was less than 80 per cent in only four instances. The usual procedure of pressing out a moist swab (used by other workers in examining eating utensils) in this work yielded numbers varying from 10 to 100 per cent, and detected on an average only 58 per cent of organisms obtainable. Since the total obtained with two swabs averaged only 83 per cent of organisms recoverable using two additional wet swabs, only pressing out one moist swab would actually yield less than 58 per cent of the organisms recoverable by four swabs. This compares with Feller's results in which he obtained 40 to 75 per cent recovery of organisms from artificially inoculated glassware using pure cultures of organisms and a smaller percentage recovery using mixed cultures. In three instances when 100 per cent were recovered by pressing out the first moist swab, there were comparatively few organisms present. In other containers the percentages obtained by pressing out the first moist swab varied considerably.

Cotton swabs are now employed in many sampling procedures, none of which specify thorough disentangling of the swab. Unless it can be certain such treatment is unnecessary in other procedures, swabs should be suspected of retaining a considerable number of bacteria difficult to remove by just moderate agitation. From the results (Table 1) it is evident that when only the contained moisture was pressed out, the swab retained, on an average, 21 per cent of organisms obtainable by the procedure used, or 26 per cent of organisms present in the swab itself. This is in accord with Dearstyne's work on cotton plugs of dilution bottles, in which he found 20 organisms per 100 were retained in cotton plugs after pressing all moisture from them.

Water blanks containing 40 c.c. were found suitable in this work since in this amount the swab could be well disentangled, thus mini-

mizing the chances for retention of bacteria. Greater amounts of diluent were not used in order that bacterial contents of containers with lower counts could be more accurately determined.

Two other methods tried in this study were unsatisfactory. By the agar-disc method it was impossible to secure an accurate count since colonies were often confluent. The rinse method was satisfactory for cylindrical containers with closely fitting tops but not for open-top rectangular containers. The procedure used seemed to overcome objections to the other methods tried. By following the moist swab with a dry one, bacteria dislodged or missed by the moist swab were picked up. This may be especially important with a paper container since it is somewhat absorbent and would have a tendency to absorb moisture and bacteria from the wet swab. It is believed that sampling each container separately was more accurate than using the same swabs over a series of containers, as has been practiced in the multiple-plate test. After swabbing a container having a high count, the organisms may be dislodged from swabs when they are wiped over succeeding boxes which may have low counts and to which the organisms might frequently adhere since the containers are of paper.

SUMMARY AND CONCLUSIONS

Swab methods were found more practical for the examination of open-top paper containers than agar-disc or rinse methods. When examined by swabbing with one moist and one dry swab, 17 quart and three pint containers, usually the top three or four in a row, gave bacterial counts ranging from 80 to 13,380 with 13 having 500 or more bacteria per container.

The usual procedure for the bacterial examination of eating utensils of only pressing out one moist swab was not satisfactory, since this recovered only 58 per cent of organisms, fluctuating from 10 to 100 per cent, recoverable by one moist and one dry swab. Thorough disentangling of the original swab recovered an additional 21 per cent of organisms actually present, showing that the ordinary cotton swab retains a considerable number of organisms after being pressed out. Use of a second dry swab recovered an additional 21 per cent of organisms from the paper containers examined.

Combining the use of one moist and one dry swab, simultaneously, breaking into a 40-c.c. dilution blank and thoroughly disentangling, proved an effective method for use on open-top paper containers. Recovery averaged 83 per cent with inoculated containers, fluctuating only between 54 and 96 per cent, and in only four of 20 examinations yielding less than 80 per cent recovery. The use of two additional moist swabs yielded 10 and seven per cent additional recovery.

Since recovery of 100 per cent of organisms would be difficult by any method, recovery of 83 per cent rather consistently by one moist and one dry swab may be considered satisfactory, and the greater effectiveness of this procedure suggests its possible application to other containers or utensils.

The results emphasize the retention of organisms in cotton swabs and the necessity of specifying conditions of disentangling in any standard procedure or standards based on swabs.

It is believed that paper containers should be sampled separately and not in multiple as suggested for eating utensils and glassware, since dislodgment and retention of bacteria by the paper container appears probable.

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NUMBERS AND TYPES OF BACTERIA ISOLATED FROM PAPER ICE CREAM CONTAINERS [1]

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(Received for publication, September 20, 1937)

Bacterial standards have been adopted by some health departments and by the industry itself for ice cream. Other requirements limiting numbers of bacteria permitted in milk cans have been applied to metal ice cream cans, and some health departments require that ice cream mix pass from pasteurizer to freezer in a continuous pipe line without exposure to handling. In some cities endeavors are made to avoid rehandling by having each individual portion of ice cream wrapped at the plant. Yet ordinarily the finished product may be handled by retailers without regard to sanitary requirements.

Paper cans are used in the ice cream industry for transportation to retailers, and smaller paper containers are extensively used for dispensing ice cream at retail stores and for packaging at the plant. This latter procedure lessens health hazards by avoiding rehandling of ice cream at retail stores, and follows the usual requirements for milk which do not permit "dipping" at the retail store but require leaving the plant and reaching the consumer in the original package.

It is generally assumed that paper containers are sanitary although few bacteriological investigations have been reported. In this study the numbers and types of bacteria in various paper containers sampled at different places were investigated. Consideration was also given to possible standards for such paper containers.

Although bacterial standards for paper containers are lacking, standards have been proposed and adopted for eating utensils, for glass milk bottles, and for metal milk and ice cream cans. A subcommittee on standard methods of the American Public Health Association (1936-1937) proposed a bacterial standard for glassware, eating utensils, and plateware stating that no utensil examined should exceed 500 organisms per utensil surface area examined. In this connection it is of interest that Cummings and Yongue (1936), during an investigation of eating-utensil sanitation, found spoons contaminated to the extent of 120,000 bacteria per spoon.

[1] Based on a portion of a thesis submitted to the Graduate School of the University of Maryland by the senior author in partial fulfillment of the requirements for the degree of Master of Science.

The International Association of Milk Dealers (1933) recommended that milk bottles not developing more than two colonies per cubic centimeter of capacity and that cans and covers not developing more than 100,000 colonies per 10-gallon can should be considered satisfactory. Parfitt (1936) stated that milk bottles and cans should not add more than one microörganism per milliliter of contents. Regulations of the Baltimore City Health Department (1936) did not allow counts to exceed 50 organisms per bottle. The U. S. Public Health Service Milk Ordinance and Code (1935) permitted one bacterium per cubic centimeter of capacity, and this is the standard generally adopted by city health departments.

Rinse methods are used to determine if milk bottles and cans meet the above standards. Standard Methods of Milk Analysis (1934) specified the use of 100 ml. of sterile water in rinsing bottles and 500 ml. in cans, the top of each container to be put on and the closed container to be shaken vigorously 25 times. The standards proposed using this method were that milk bottles should not develop over one colony per milliliter of capacity, and cans and their covers not over 100,000 colonies per 40-quart can, 50,000 per 20-quart can, or 25,000 per 10-quart can. In a report of the American Public Health Association (1933-1934) it was stated that 56.9 per cent of bottles examined and 37.7 per cent of cans examined complied with the standard of not over one bacterium per cubic centimeter of capacity. There was no definite recommendation as to whether such a standard was too rigid or not rigid enough, and the suggestion was made that the problem be studied fundamentally.

Fabian (1935) reported a definite trend toward more adequate state and city sanitary regulations governing ice cream production. At that time twelve states had established bacteriological standards. Horn (1935) studied the contamination of ice cream dippers in retail stores and found ice cream of a good quality originally may be grossly contaminated by rinse water adhering to the dipper used to serve it. It is of interest that the most contaminated water was found in pharmacies having, of course, a registered pharmacist. After instituting an educational program, a definite betterment of such conditions was noticed.

Bayer (1932) discussed the use of paper cans by ice cream plants and pointed out that they had a very desirable effect in plant operation from the standpoint of sanitation and improvement of appearance by elimination of dirty cans and can-washing equipment. Bayer (1931-1933), using a rinse method for sampling, found an average of 425 organisms in 10 paper ice cream cans which had been in a stockroom about three months. He considered these counts appreciably

lower than the usual counts of metal cans and termed them "bacteriologically clean" and almost sterile.

Stewart (1904), using a rinse method, found no evidence of contamination in a number of paper milk bottles examined. He attributed this condition to the hot paraffin bath followed by subsequent baking, which apparently destroyed all organisms present on the paper. He also found milk kept better in these containers than in glass bottles.

Rice (1930, 1932) found paper bottle caps may be a highly potential source of contamination from which bacteria may enter the bottled milk. Later work presented evidence that these bacteria originated in the paper manufacturing plant; after obtaining samples he definitely showed that water used in the process was the chief source of organisms, especially of the intestinal bacteria, found in the finished capboard. Schooley (1936) identified bacteria isolated from milk-bottle caps obtained from the manufacturer. The organisms studied were all gram-positive, spore-forming rods, and with few exceptions were placed in the 14 species of Bacillus identified. No correlation could be made between the geographical location of the manufacturing plant and the types of organisms isolated.

Frier (1937) stressed the necessity of using containers made from "the highest quality paper," declaring that a cheap package will make the best ice cream cheap. The Baltimore City Health Department adopted a regulation (1937) governing paper, cardboard, and other non-glass containers to be used in milk plants, requiring that paper blanks or non-paraffined containers be received by the milk plant in tightly closed packages or cartons and be manufactured from the best obtainable white spruce pulp or other material approved by the Commissioner of Health. The container, if it required paraffining, had to be paraffined in the milk plant where it was to be filled with milk or milk products; and other requirements specified that the containers be so handled as to prevent hand contamination.

EXPERIMENTAL PROCEDURE

Swabs used in this work consisted of cotton wound on the end of a six and one-half inch wooden applicator to a thickness of one-quarter inch and breadth of one inch (those used for the ice cream dishes were one-third this size). The swabs were wrapped in pairs in brown paper and sterilized by autoclaving at 120°C.(248°F.) for 30 minutes, as were the dilution blanks which contained 40 c.c. of distilled water (or 20 c.c. for the ice cream plates). Standard nutrient agar adjusted to a pH of 7.0 was used for plating unless otherwise designated. Plates were poured in duplicate and incubated at 37°C.(98.6°F.) for 48 hours.

The following procedure was used since this had proved effective in a previous comparison of possible methods. Two swabs held in the same hand, the first moistened from the 40-c.c. water blank used and the second dry, were used to swab the inside of the container thoroughly. Both swabs were broken into the 40-c.c. dilution blank and shaken until disentangled, when one-c.c. aliquots were plated in duplicate. The average counts were reported as bacteria per container.

Containers were secured from a number of local restaurants and drug stores. Fifteen containers, obtained in a nearby city from each of two ice cream plants which acted as wholesale distributors for most of the containers sampled at retail places, were taken from previously unbroken packages as received from the manufacturer in an effort to determine if any contamination was traceable to the manufacturer. Some similar open-top containers used for dispensing other foods, such as oysters and sauerkraut, were obtained from various grocery stores in order to compare them with paper containers used for dairy products.

Three different types of boxes and various sizes of each were examined. Most of these were of the open style having four folding flaps to close the top. In the retail store these were lying in rows, or nests, the top one being exposed directly or indirectly, depending on storage conditions, to the environment of the store. Those obtained at grocery stores were of a similar style although their construction was different and they were paraffined. The other type examined was cylindrical, with a tightly fitting crown top. In addition to these, a small number of paraffined paper ice cream dishes were obtained from a local dairy. At the time the open-top containers were obtained the manner in which they were stored was recorded as "poor" designating exposure directly to air and dust of the store, "fair" indicating some protection in a loosely fitting cabinet or cardboard box, and "good" referring to storage in a tight cabinet well protected from the dust and air of the store.

In order to obtain information regarding the type of bacteria present, 11 strains of organisms were isolated from plates from containers obtained in retail establishments. Seven strains were also isolated from plates from containers sampled at ice cream plant No. II as they were received from the paper manufacturer. These organisms did not constitute the entire flora of these containers, but were selected as the predominating organisms in each instance. The technique and media employed in identifying these organisms were those recommended in the Manual of Methods for Pure Culture Study of Bacteria (1934).

In another series of containers sampled, plates were also poured with tryptone glucose skim milk agar and tubes of two-per cent brilliant green lactose peptone bile inoculated.

RESULTS OBTAINED

The results show that the bacterial content of the box located at the top of a row or nest was almost always higher than at any other location in the row (Table 1). The number of bacteria found in the paper containers usually varied directly with the storage conditions, high counts being found where storage conditions were poor. Counts of containers originally from the same wholesale dealer also varied at retail dealers according to their storage conditions.

There was no direct correlation between the count and location of containers beneath the top one, although usually counts were decidedly lower than the top container. There were several exceptions for which there was no apparent explanation unless such containers may at one time have been exposed in the top of a row which was later combined with another, these containers then being beneath the top. Although these boxes fit together very tightly, it is also possible that the exposed flaps of the boxes permit bacteria to collect in the top of the box.

Storage conditions at retail establishment No. 9 were exceptionally poor. The containers were stored on shelves which were close to the floor in cabinets with sliding doors which were seldom closed. This direct exposure to dust and dirt from the floor, particularly that due to continued passage of clerks, undoubtedly accounted for the exceptionally high counts found there; since at other establishments where storage conditions were poor, containers were never so close to the floor and their counts were usually much lower.

For a comparison of different types of paper containers, cylindrical containers with close-fitting crown tops were obtained from various sources. The inner surfaces of these containers were protected at all times from exposure to the environment in which placed, and results of this protection are seen in their consistently low counts (Table 2). It is our understanding that these containers are sterilized during manufacture.

The consistently negative results obtained on paraffined paper ice cream dishes was of particular interest. Since 20-c.c. dilution blanks were used and plates poured with one c.c. in duplicate, there must have been less than 10 bacteria per dish. However, one is led to conclude that these dishes must have been manufactured, shipped, and stored under excellent conditions. At the ice cream bar the dishes were inverted in a wire support so dust could not collect on the

TABLE 1

Bacterial Content of Open-Top Paper Ice Cream Containers From Various Retail Stores

(Counts expressed as colonies per container)

Sample collected	Dealer		Storage conditions	Size	Location in stack				
	R.[1]	W.[2]			Top	2nd.	3rd.	Last	Other
12/28/36	2	III	Good	Quart	200	60	80	....	....
12/28/36	....	....	Good	Pint	0	60	0	....	....
4/ ?/37	6	IV	Good	Half-pint	0	80	0	....	....
4/ ?/37	4	II	Fair	Half-pint	200	60	120	....	....
11/20/36	7	I	Fair	Pint	0	....	....	0	40
12/ 4/36	7	....	Fair	Pint	0	0	....	....	....
12/ 4/36	....	....	Fair	Pint	0	0	....	....	....
3/ 3/37	7	....	Fair	Half-pint	0	60	400	....	0
3/ 3/37	....	....	Fair	Half-pint	....	....	....	....	100
12/28/36	1	II	Poor	Quart	600	840	740	....	....
12/28/36	....	....	Poor	Pint	280	160	80	....	....
12/28/36	3	I	Poor	Quart	420	300	1,050	....	....
12/28/36	....	....	Poor	Pint	240	128,800	40	....	....
4/ ?/37	5	II	Poor	Half-pint	3,200	0	40	....	....
4/ ?/37	....	....	Poor	Half-pint	980	....	....	....	....
12/19/36	8	....	Poor	Quart	4,440	200	240	....	....
12/ 3/36	8	....	Poor	Pint	1,920	80	....	....	1,650
12/ 3/36	....	....	Poor	Pint	540	800	....	....	980
12/ 3/36	....	....	Poor	Pint	80	0	....	....	....

TABLE 1 *(Concluded)*

11/16/36	8	....	Poor	Pint	100	40	....	....	....
11/16/36	....	....	Poor	Pint	60	....	....	....	....
3/ 3/37	8	....	Poor	Half-pint	6,080	80	160	....	....
11/16/36	9	I	Poor	Quart	8,320	....	....	400	180
11/20/36	9	....	Poor	Quart	16,400	....	....	900	....
11/20/36	....	....	Poor	Quart	19,240	....	....	....	....
12/19/36	9	....	Poor	Quart	5,180	80	500 a, b	....	2,340 e
12/19/36	....	....	Poor	Quart	18,380	1,600	480	....	....
12/19/36	....	....	Poor	Quart	480	60	80	....	....
12/ 1/36	9	....	Poor	Pint	60	120	....	0	....
12/ 1/36	....	....	Poor	Pint	0	60	....	40	....
12/19/36	9	....	Poor	Pint	520	260	11,680 b, e	....	....
3/ 3/37	9	....	Poor	Half-pint	1,420	240	0	....	....
12/ 4/36	9	....	Poor	Quart	91,000	....	....	....	....
12/ 8/36	9	....	Poor	Quart	600 g[3]	....	....	....	....
12/ 8/36	....	....	Poor	Quart	6,600	....	....	....	....
12/ 8/36	....	....	Poor	Quart	1,680 e, i, f	....	....	....	....
12/ 8/36	....	....	Poor	Quart	3,600 k	....	....	....	....
2/13/37	9	....	Poor	Quart	4,160	....	....	....	....
2/13/37	....	....	Poor	Quart	7,320	....	....	....	....
2/25/37	9	....	Poor	Quart	8,200	....	....	....	....
2/25/37	....	....	Poor	Quart	11,160	....	....	....	....

[1] Retail. [2] Wholesale. [3] a—*Micrococcus conglomeratus*; b—*Micrococcus luteus*; c—*Micrococcus nitrificans*; f—*Micrococcus varians*; g—*Sarcina flava*; i—*Micrococcus cheronesia*; k—*Sarcina hamaguchii*.

inside surface of the container, a factor undoubtedly influential in maintaining a low count.

The bacterial counts of paper containers as used in grocery stores for dispensing other foods, such as oysters or sauerkraut, were also studied. These containers were not stored in any manner which would

TABLE 2

Bacterial Content of Various Paper Containers

(Counts expressed as colonies per container)

Sample	Cylindrical containers with close-fitting tops			Paraffined ice cream dishes		Open-top containers from grocery stores	
	Quart	Pint	Half-pint	Series 1	Series 2	Quart	Pint
1	80	60	120	0	0	1,640	600
2	0	0	0[a]	0	0	1,080	940
3	0	0	100	0	0	620	400
4	80	0	40	0	0	4,900	120
5	0	40	60	0	0	7,160	0
6	0	80	40	0	0	360	100
7	40	0	60	0	0	10,580	2,400
8	0	80	0	0	0	2,960	40
9	0	60	0	0	0	2,060	1,540

protect them from the environment and were sometimes placed behind the counter close to the floor, where any dust stirred up by clerks could easily fall into them. This is probably the main reason for their comparatively high counts, and on this basis they present a problem similar to that of containers used for dairy products.

TABLE 3

Bacterial Content of Paper Containers Directly From Ice Cream Plants

(Counts expressed as colonies per container)

Plant I			Plant II		
Quart	Pint	Half-pint	Quart	Pint	Half-pint
0	100	0	500	960 d	40
0	80	80	620 d[1]	960 d, h	180
0	0	40	760 e	430	120
0	0	0	80 h, j	160	180
0	0	0	140	260	140

[1] d—*Alkaligenes fecalis*; e— *Achromobacter* species; h—*Micrococcus nitrificans*; j—*Bacillus albolactis*.

The role that the paper manufacturer may have in the initial contamination of containers is indicated (Table 3). Containers from Plant I had a consistently low count and in many of them no bacteria were found in the dilution used (1/40). Containers from Plant II contained relatively many organisms, although the one-half-pint size

TABLE 4

Bacterial Content of Open-Top Paper Ice Cream Containers From Various Retail Stores, Comparing Tryptone Glucose Skim Milk Agar With Standard Agar and Including Presumptive Test for Coliform Bacteria

Dealer		Storage conditions	Size	Location in stack					
				Top			Second from top		
				Agar		Broth [2]	Agar		Broth [2]
Retail	Wholesale			Standard	Tryptone		Standard	Tryptone	
9	I	Poor	Quart	15,360	6,160	0	1,660	1,000 [1]	0
9	I	Poor	Quart	8,600	5,400	0	520 [1]	360 [1]	0
9	I	Poor	Pint	1,000 [1]	440 [1]	0	80 [1]	120 [1]	0
9	I	Poor	Half-pint	1,840	1,760	0	400 [1]	280 [1]	0
9	I	Poor	Half-pint	800 [1]	520 [1]	0	80 [1]	240 [1]	0
8	II	Poor	Quart	1,100 [1]	1,300	0	280 [1]	200 [1]	0
8	II	Poor	Pint	6,840	5,680	0	7,160	4,960	0
8	II	Poor	Half-pint	1,360	520 [1]	0	1,360	500 [1]	0
5	II	Poor	Quart	600 [1]	1,140 [1]	0	440 [1]	540 [1]	0
5	II	Poor	Half-pint	4,080	5,800	0	1,560	1,160 [1]	0
4	II	Poor	Pint	1,320	520 [1]	0	1,240	440 [1]	0
4	II	Poor	Half-pint	1,160 [1]	920 [1]	0	840 [1]	960 [1]	0
Average	....	....	....	3,670	2,515	....	1,300	895	....

[1] Less than 30 colonies per plate. [2] Presumptive test for coliform bacteria using two-per cent brilliant green lactose peptone bile, 0 meaning no gas formation.

did not contain as many as larger sizes. The typical contaminants of these containers were chiefly organisms normally present in pollution.

Standard methods agar was a suitable medium for plate counts of organisms on the paper containers (Table 4). The use of tryptone glucose skim milk agar did not yield increased counts over standard methods when plates were poured from the same dilution blanks, but averaged only 1,705 colonies as compared with 2,480 with standard

TABLE 5

Summary of Number of Bacteria Obtained in All Paper Containers

Type	Place	Series	Colonies per container						Total
			0-20[1]	21-100	101-200	201-500	501-1,000	Over 1,000	
Open-top	Retail	Series I							
		Top	7	4	2	4	5	18	40
		2nd.	4	11	3	3	2	2	25
		3rd.	3	5	2	4	1	2	17
		Last	2	1	0	1	1	0	5
		Other	1	2	1	0	1	2	7
		Series I Total	17	23	8	12	10	24	94
Open-top	Retail	Series II[2]							
		Top	0	0	0	0	2	10	12
		2nd.	0	2	0	3	2	5	12
		Series II Total	0	2	0	3	4	15	24
		Total of Series I and II	18	23	8	16	14	39	118
Open-top	Plant I		11	4	0	0	0	0	15
Open-top	Plant II		0	2	6	3	4	0	15
Open-top	Grocery stores		1	2	1	2	3	9	18
Cylindrical	Retail		13	13	1	0	0	0	27

[1]One colony on one duplicate plate of 1-c.c. (swabs rinsed in 40-c.c.). [2]Sampled only from poor storage conditions.

agar on the total containers compared. This series of two dozen containers from four retail establishments representing containers from two wholesale plants was entirely negative for members of the coliform group when one cubic centimeter was inoculated in duplicate into two-per cent brilliant green broth. Since this inoculum was five per cent of the total volume of water in which the swabs were rinsed, it indicates the relative freedom of the containers from coliform bacteria. All bacterial counts obtained are summarized (Table 5).

DISCUSSION

It is apparent that storage conditions in retail stores are important in maintaining a low bacterial count of paper containers. Boxes at Plant I, as they came from the manufacturer, had a consistently low count; but when obtained at retail stores counts usually increased, the extent being determined by the storage conditions. Since in poor storage conditions the containers were always exposed directly in the store, one answer to the problem might be storage in tight cabinets, inverting the rows with the tops downward, and then using the top box of the inverted rows when a product is to be dispensed. This would also avoid contaminating the inside of the container by the hands of the clerk, which inevitably occurs when rows of boxes are kept in an upright position and the top box is used. Another solution may be the use of cylindrical containers with tightly fitting crown tops protecting the inside from contamination from the time of manufacture until used.

That the paper manufacturer may be responsible for some contamination is indicated by the comparatively consistent counts of containers at Plant II as received from the manufacturer. These containers were sampled at two different times with about a month intervening, and it is logical to conclude that the results represented the normal bacteriological condition of these containers.

Counts on the open-top rectangular box, especially those exposed directly, indicate a public-health problem. A comparison of counts obtained with standards for milk bottles reveals that if such standards were to apply to paper containers, many would far exceed the maximum allowable count generally prevailing for milk bottles of 1,000 per quart, 500 per pint, and 250 per half pint. These standards are quite lenient compared with one adopted by the Baltimore City Health Department which does not permit the count per bottle to exceed 50 organisms, a standard only a few of the paper containers could meet. Translating the maximum allowable count of 500 organisms per four square inches, proposed for plateware, into its equivalent in bacteria per container, the approximate maximum counts permitted would be 10,000 per quart, 6,500 per pint, and 4,000 per half-pint container, considerably more lenient counts than generally are in effect for milk bottles. Yet, if the method suggested by the subcommittee proposing this standard be used, in which one swab is only agitated in the diluent, recovery of approximately only 58 per cent of the bacteria actually present could be expected, according to other results obtained, Speck and Black (1937).

Bacterial counts of paper ice cream containers examined in this investigation were, as a whole, considerably higher than those found

by Bayer in paper cans, especially when the total surface area sampled in the two different types is considered.

The organisms isolated from the various containers are of interest since all strains except one were non-spore-formers, differing from Schooley's findings in which organisms isolated from milk-bottle caps were all gram-positive spore-formers. One would expect more spore-formers if the entire microbial flora of the containers had been studied, although the organisms isolated were predominating in that container. Of these, some were natural inhabitants of air, water, and milk, and these were the chief contaminants of containers exposed in retail stores. Most organisms isolated from containers directly from the paper manufacturer were types that are naturally found in the intestinal tract and sewage, indicating the containers were contaminated by some source of pollution. These results are similar to those of Rice on intestinal bacteria found in finished capboard and make it logical to suspect the manufacturer of containers for Plant II of possibly using a polluted supply of water in his process. The evidence presented would suggest a more detailed study of the bacteriological condition of containers from various manufacturers and of materials used in their manufacture.

SUMMARY AND CONCLUSIONS

The bacterial counts of open-top ice cream containers obtained from retail establishments varied widely, and apparently correlated with the storage conditions in the retail stores. Contamination from air and dust probably were the most important factors influencing these counts.

Cylindrical containers with close-fitting crown tops had the most consistently low counts of any paper containers examined, except the paper dishes.

An important factor influencing the low or negative counts of paper ice cream dishes undoubtedly was the manner in which they were inverted at the ice cream bar until used. By storing ice cream boxes in a similar manner, using first the top box of the inverted row when dispensing a product, the counts of these containers should be kept at a minimum, provided they were received from the paper manufacturer in a good sanitary condition.

That the paper manufacturer may be responsible for some of the contamination was shown by counts of containers from one ice cream plant where containers were sampled as they came from the manufacturer. Such a condition was not found in containers of a different brand sampled at another plant, and it would seem manufacturers of paper containers for food products should control contamination

due to manufacture by elimination or by sterilization of the finished product.

Some typical organisms contaminating paper containers isolated were identified as members of the genera Micrococci (11 strains), Alkaligenes (3), Sarcina (2), Bacillus (1), and Achromobacter (1). The presence of some of these strains in paper containers secured directly from the manufacturer suggested contamination by sewage pollution.

Inoculations were made from one group of containers into brilliant green lactose peptone bile using five per cent of the liquid in which the swabs were disentangled, and the consistently negative results indicate their relative freedom from coliform bacteria. With this same group of containers the use of tryptone glucose skim milk agar for plating resulted in lower counts than with standard methods agar which had been used throughout this study.

Applying the standards for milk bottles and other dairy utensils of one organism per milliliter of capacity, would disqualify many of the containers examined. Application of the proposed standard for plateware of 500 organisms per four square inches of surface would perhaps be too lenient for public welfare; however, standards for paper containers and a standard method for sampling should be adopted.

In order to eliminate entirely the factor of probable contamination by the retail merchandiser, a logical procedure would be to have ice cream wrapped or packed in the final package at the ice cream plant, a requirement similar to that for other dairy products, such as milk.

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REVIEW OF LITERATURE ON MEAT FOR 1936

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(Received for publication, September 22, 1937)

PLANT OPERATIONS—SLAUGHTERING, STORAGE, AND BY-PRODUCTS

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Innovations in the mechanical and physical phases of the meat-packing industry are a matter of keeping in step with the general progress displayed in all industries; for instance, improved air-conditioning equipment has been designed for various packing-house uses; two of these are described by Gelliam (1936). Moulton (1936) described current methods for several packing-house operations. These papers dealt with killing and cutting the animals, processing the various parts of the viscera, and curing the meat.

Callow (1936) found that the electrical resistance of muscular tissues of farm-killed pigs was lower than that of pigs slaughtered at a packing house. It was suggested that the difference was due to the effect of transportation and overheating during the drive to the slaughtering site. Low electrical resistance in the meat was associated with low moisture loss and with greater salt permeability during curing. This author also substantiated a popular belief that fasting pigs before slaughter increases the thickness of the flank. Data on the above work and the effect of growth on the chemical composition of the tissues and the effect of growth rate on the quality of the carcass were presented both graphically and in tabulated form. It is interesting to note that fat content of the fatty tissue showed a well-marked increase with advancing age of animals and that the faster the rate at which fat was deposited in the fatty tissues the more saturated the fat became.

Meat from boars and recently castrated boars (stags) gives off a very unpleasant odor during cooking or roasting. In the literature this is referred to as sexual odor; technicians at the packing plants usually refer to the meat as being "staggy." Several German investigators studied this problem with the aim of devising a simple test to detect "stagginess" so that such meat can be identified and diverted to processing rather than be marketed. Gerche (1936) reported that in castrates the parotid gland was the most potent carrier of the odor. He recommended that a cooking or roasting test be made on this gland from carcasses which are suspected of carrying the odor. A negative test would be assurance that the carcass fat or meat

will not give the "staggy" odor during cooking or baking. A weakly positive test should be followed by a similar test on a sample of fatty tissue from the carcass; if this test is negative, even though that on the parotid gland is slightly positive, the meat can be considered marketable. Keller (1936) stated that the testes from a boar's carcass gave the most potent odor in the test followed by submaxillary glands, parotid and sublingual glands, fat, and flesh. The submaxillary gland from recent castrates was, therefore, considered the best material for the test, but he recommended that all the salivary glands be used, thus giving more test material. In order to determine how long after castration "stagginess" prevails Lerche (1936) experimented on 32 boars. They were castrated, and slaughtering of the animals was extended periodically from eight to 75 days after castration. "Stagginess" disappeared from the flesh about the tenth day after castration; at 30 days the test was slightly positive in the fat and not entirely negative until after 68 days. A positive test was still obtainable with the parotid gland after the tests on the flesh and fat were already negative. Heydt (1936) and Kunze (1936) recommended diverting suspected meat to processing rather than risking attempts to market it.

Literature pertaining to storage of meat in an atmosphere containing carbon dioxide was reviewed by Fisk (1936). Alekseev *et al.* (1935) reported tests on the effect of carbon dioxide on foods. The period of conservation of flesh foods was lengthened by the gas. With fish an off-color was produced; meat, however, retained its color better. A patent was issued to Ryan (1935) on using the stack gases obtained from ship funnels as the gas for the preserving atmosphere during ship transportation. The composition of the gas should be not more than seven per cent of oxygen, carbon dioxide 10 to 14 per cent, and the remainder nitrogen. The gas is precooled to a temperature below that at which the meat is to be kept. According to Stansby and Griffiths (1935), fish stored in carbon dioxide kept approximately twice as long as controls stored in air. Experiments on fillets indicated that the use of hypochlorite solution offset the effect of careless handling and benefited by the use of carbon dioxide. Two patents on disinfecting flesh foods before storage in carbon dioxide atmosphere were issued to Möller and Peterson (1935). The patentees recommended hydrogen peroxide as the disinfecting agent. Results of tests on carbon dioxide storage of bacon by Callow (1936a) added new information which favored gas storage. Bacons stored seven months in carbon dioxide yielded negative tests for rancidity, while controls stored in air were rancid after four months' storage. The controls, i.e., those stored in air, lost considerable weight.

Ozone has also received consideration in the storage of foods. Ewell (1936) claimed that the growth of mold on meat was prevented by maintaining an ozone concentration of about one and a half parts per million in the storage room atmosphere and that high concentration of ozone (50 p.p.m.) was injurious to fats and foods containing fats.

Evaporation of moisture from meat in storage was investigated by Tuchschneid (1935) and Hicks (1936). Tuchschneid showed that during six months' storage, beef lost 1.9 to 3.7 per cent of its weight, mutton 2.5 to 3, fish 1.32 to 1.9, poultry .91 to 1.85 per cent. Data included temperature and humidity of storage atmospheres for the various products. Hicks showed that rates of evaporation from surfaces cut parallel to the muscle fibres, surfaces cut normal to the fibres, and natural surface of muscle freed from tissue were the same. Measurements were also made on the water content of meat at different depths below the surface for various durations and rates of evaporation. It seemed that during evaporation there was a diffusion of crystalloids away from the surface as well as a diffusion of water towards the surface.

The "Murphy system" of quick freezing was described anonymously (1936). Haslacher (1936) also described a method of quick freezing. Both descriptions pertained to mechanical features used in the process. Heiss (1936) mathematically evaluated the most favorable freezing conditions for meat. His conclusions were as follows: (a) Freezing should take place as soon as possible after killing because on standing, the pH drops; a low pH value unfavorably influences freezing changes. (b) Freezing should be performed as fast as possible and the freezing temperature should be below —17°C. (1.4°F.). A method of improving the color of frozen meat was originated by McKee and Henney (1936). A desirable bright red color was induced in meat by subjecting it to pressure while at a temperature of —3.9 to 0.0°C.(25 to 32°F.). In place of pressure, the meat may be comminuted at the above temperatures.

The development of what is known as "freezer burns" is a serious injury to stored and frozen meats. As cited in a publication by Tressler (1935), freezer burns are caused by uneven desiccation of stored poultry or other meats; simultaneous changes may also occur in fats and proteins of the tissues underneath the dried areas. The proteins become denatured, and the lost water cannot be again absorbed. Dipping poultry or meat in water before freezing or storage in an atmosphere of carbon dioxide reduces freezer burns.

Experiments on the rapidity of decomposition of thawed and fresh meats were reported by Marginescu (1935). Meat that had been

frozen for three months and then thawed decomposed less rapidly than fresh meats that were kept frozen for more than six months. Any of the frozen meats that were thawed rapidly decomposed sooner than fresh meats or meats that were thawed progressively. A method involving temperature and humidity control for thawing meat progressively with prevention of weight loss by water evaporation was described by McKee and McDonald (1936).

Several patents were issued on curing salts for meats. Those by Griffith and those assigned to the Griffith Laboratories, Inc. (1936) dealt with fusing various inorganic salt-curing mixtures and use of the fused salts in curing. In common curing practices sodium and potassium salts are used. According to two recent inventions by Metzner (1936) and Genin and Blanckaert (1935), additions of ammonium salts improve the curing. The last-mentioned invention also includes addition of phosphate salts to the curing mixture; this was said to reduce the time necessary for curing. Hall (1935) recommended addition of .85 per cent of citric acid to the meat-pickling brine for accelerating conversion of sodium nitrite to nitrous acid, the latter being more reactive with hemoglobin and thus accelerating the cure. To improve the flavor of cured products, Allen (1936) added sodium glutamate to the curing mixture.

Methods of treating cured meats to preserve them during storage were presented by Gören (1936) and Francioni (1935). Gören dipped the unsmoked articles in an emulsion prepared by mixing disintegrated animal-skin waste with hot aqueous salt solution and with castor oil and glycerol. Articles were then dried so that a protective film formed on them and finally smoked with smoking powder which contained formaldehyde. Francioni reported that treating smoked meat, prior to storing, either with 55 per cent alcohol or with dipping in hot water and allowing the meat to dry, reduced mold formation. Mold growth was effectively prevented by storing the meat in oil.

Data on the penetration and distribution of salt in ham during curing was tabulated by Miller and Ziegler (1936a). In all curing methods used, it was found that aging of hams for 30 days after cure permitted further equalization of the salt content and gave products of more uniform salt content. A patent assigned to Musher (1936) described a method of preventing oxidation of fat of cured meats by dusting with an antioxidant material, such as pulverized oats.

Several patents were issued on the preservation and treatment of whale flesh. Various methods of drying and preserving the meat were claimed by Nygaard (1936), Fauth and Reichert (1936), Pehrson and Pehrson (1936), Bostock (1936), Hansen (1935), and Hildisch (1936).

Walter (1936), Schenk (1936), and Atkinson (1936) patented improvements in manufacture and uses of artificial sausage casings prepared from cellulose. Becker (1936a) and Freudenberg (1936a) prepared artificial sausage casings from hides. The hides were defatted, converted by swelling chemicals to a plastic, and extruded to the desired shape. An ink prepared by Walter (1936b) for marking natural casings contains a protein precipitant, such as alum, in addition to the pigment and the fluid vehicle.

Cox (1936) reviewed the trend in composition of meat extracts for the years 1895 to 1936. Data were tabulated. During recent years some of the products have contained added starch, gelatin, and soybean meal. The need of standard requirements for composition was emphasized. Methods for preparing meat extracts have been patented by Darling (1936) and Schmalfuss and Werner (1935). Darling's patent dealt with the control of pH during extraction with water. Schmalfuss and Werner added water to the meat and heated the mixture at 26.7 to 35.6°C. (80 to 96°F.); the liquid was next centrifuged from the solid and then concentrated in vacuum.

ANALYTICAL METHODS FOR ADULTERANTS, CURING SALTS, AND METALS

M. M. Piskur

Adulteration of meat products is easiest with sausage. Addition of non-flesh material, except condiments and curing salts, is sometimes made to give special qualities to the product; in some countries, when the added materials are declared on the meat or packages, it is not considered adulteration. When added to improve texture and physical appearance, they are called binding agents. A comprehensive review of them has been prepared by Lindner (1936).

In recent years addition of proteins other than meat proteins has been a common practice. Owing to convenience and to sales efforts by manufacturers soybean flour and skim milk seem to have predominated in this field. A report by Kerr (1936) described in detail procedures for detecting these substances and also the limitations of the methods. One method for detection of soybean flour was based on liberation of ammonia by urease; because the test is negative when soybean flour used in the sausage has been preheated to destroy the enzyme, microscopic identification of soybean cells may be necessary also. The latter method was described. The detection of dried skim milk was based on conversion of lactose to lactose-osazone and its identification by melting-point determination.

Another possible adulteration of meat products is addition of sodium glutamate to meat broth. An investigation by Englis and Friedman (1935) showed that detection of this substance by precipitation of zinc glutamate was subject to interference by other constituents of the broth. The ratio of alkalinity in the ash of the broth to the total ash was less significant than the alkaline value alone.

Analytical methods reported for determining nitrite and nitrate in meat or meat pickle were descriptions of known methods or slight modifications of old methods. Jones (1936) described both the McVey method for estimation of nitrates plus nitrites in cured meat and the Ilosovay methods for nitrites. Woidich (1936) recommended use of the photometer in colorimetric methods for these salts. Detailed description for determining nitrite in meat-pickle solution by permanganate titration was described by Stamm (1936). The method described was said to be more accurate than those heretofore described for solutions containing salt and sugar.

Banfield and Callow (1935a and 1935b) reported investigations on a nonchemical technic for determining salt penetration and amount of salt in cured meat without mutilation and consequent loss of meat. Measurements of electrical resistance made with a two-prong probe insulated to the tips on standardized pieces of meat were checked by analysis for salt and the results plotted. At the same salt concentration, fat cuts had greater electrical resistance than lean cuts, minced less than intact pieces of pork, and lean pieces showed greater resistance across the fibres than with them. Moisture and fat content of muscle tissues also influenced the resistance of the lean portion of meat. Various data and charts indicated that electrical resistance measurements can be used for control work in the curing of meat.

Greater effort on the part of food and drug officials has been made toward enforcing the laws pertaining to the lead content of canned meats. Lead may be picked up by meat from processing equipment, tin plate, and solder used on the cans; in addition small traces are occasionally naturally present in the animal tissue. Methods for determining lead in flesh foods were reviewed by Schirokow and Mindlina (1935). The dithizone method for determining lead was described by Fabre and Lem (1936) and Clifford and Wichman (1936). Clifford and Wichman classified methods using dithizone and indicated the limitation and errors possible by each of them. A photometric method of measuring the color, which increases the sensitivity and accuracy of the dithizone methods for lead, was also presented.

POST-MORTEM CHANGES—AGING, ENZYMES, BACTERIOLOGY, AND FOOD POISONING

J. M. RAMSBOTTOM

Physiology of muscle protein with particular reference to changes during rigor mortis has been investigated by Smith (1935). During the actual process of stiffening he found that no changes in the properties of myosin can be detected nor is there any appreciable decrease in the salt extractable fraction of the proteins.

Maturing of meat consists of transformation of active albuminoids of the muscle into coagulated substance, according to Smorodintsev (1936). Chemical indices for matured meat were given by the author as follows: (1) .7-.8 per cent lactic acid, (2) .2 per cent reducing substance, (3) pH 5.6-5.7, (4) ratio amino nitrogen: reducing substances .25-.27, (5) ratio glycogen: lactic acid .2, (6) ratio ammonia: total nitrogen .008-.009. Maturing of meat required from 24 to 72 hours and depended on the individual properties of the meat and on the temperature of the environment.

Smorodintsev and Laskowskaja (1935a) reported experiments on the determination of digestibility of meat protein by use of pancreatin. They claimed that maximum digestibility of meat was found in the sixth hour after slaughter and was independent of the temperature at which it was held. The mean increase in digestibility was constant for different animals. Increase in the storage temperature of meat from 1 to 3°C.(33.8 to 37.4°F.) up to 37°C.(98.6°F.) accelerated protein changes but in no way altered the character of the curve. Somewhat different results were obtained by the same investigators (1935b) when pepsin was used in place of pancreatin in digestion experiments on meat proteins. Pepsin digestion showed that lean beef reached its maximum digestibility in 96 to 120 hours after killing when stored at 1 to 3°C. or in 6 to 24 hours when kept at 36°C.(96.8°F.).

Smorodintsev and Adova (1935) reported that collagen fibers required 24 hours or three times as long as pure muscle fibers for digestion with 1:100 pepsin solution. Thus, high collagen content in meat decreased the digestibility.

Modification of proteins in meat by its own enzymes has been studied by Sadikov and Shoshin (1936a). They stated that at 17°C. (62.6°F.) slight alteration of globulins occurred in two days and marked alteration in 27 days . At 37°C.(98.6°F.) there was extensive peptone formation over periods up to four months, and at 55 to 60°C. (131 to 140°F.) the breakdown proceeded in a few days to amino acids. In all tests toluene was used to stop bacterial action. They

believed that ripening of stored meat is not autolytic but a process equivalent to enzyme stabilization with conversion of globulin to globulan-insoluble in 10 per cent sodium chloride and 20 per cent hydrochloric acid solution. The same investigators (1936b) reported that meat stored in carbon disulfide vapor is acted upon by its own proteolytic enzymes but not by bacterial enzymes. In carbon disulfide storage a conversion of meat globulins to salt insoluble globulan occurred and slight degradation of albumins to peptones. Unlike strong antiseptics carbon disulfide vapor did not have denaturing effect on meat proteins.

Mirsky (1936a) reported that protein coagulation produced by denaturing agents was different from coagulation of myosin by the unknown agent in muscle since, denaturing agents activated SH groups, whereas the formation of insoluble protein by rigor was not accompanied by the activation of SH groups. Study of sulfhydryl and disulphide groups of proteins by Mirsky (1936b) revealed that SH groups of native muscle proteins are activated by a rise in pH. Smorodintsev and Tgiganova (1935) stated that changes in total, inorganic, and organic sulphur and in SH group were not well enough defined to characterize the different stages of muscle autolysis.

Study of the dynamics of extractive substances in autolyzing muscle tissue was made by Smorodintsev and Nikvlaeva (1935). They contended that raising the temperature of muscle tissue from 4 to 36°C.(39.2 to 96.8°F.) did not alter the general direction of the curve of formation of muscle extractives.

Smorodintsev and Filippova (1935) claimed that splitting of muscle glycogen, which occurred rapidly in the beginning of autolysis, showed up gradually; and at 4°C. from the fifth to the tenth day and at 36°C. from the third day glycogen was resynthesized, the resynthesized glycogen reaching 50 to 80 per cent of that present at the beginning. In the same paper they stated that inorganic phosphorus in muscle-tissue extractives remained constant in the first hour of autolysis at .07 per cent. Increase in inorganic phosphorus in the extract ran parallel to the increase in glycogen. From correlation of the above factors with the consistency of meat, they believe the process of ripening occurred in the period from the 24th to the 120th hour.

Changes in solubility of calcium compounds during autolysis of beef-muscle tissues was studied by Smorodintsev and Laskowskaja (1935c). One hour after slaughter 30 per cent of the calcium was extracted by water; after storage for 24 and 72 hours at 1 to 4°C. (33.8 to 39.2°F.) 57 and 62 per cent of the calcium, respectively, was

extracted. Increase in solubility of calcium paralleled the formation of lactic acid in the muscle.

Sadikov and Starukhina (1936) made a number of experiments on thermal denaturing of meat. When meat was heated from 85 to 120°C. (185 to 248°F.), loss of water-soluble proteins ranged from 50 to 70 per cent. Sadikov and Khaletzkaya (1936) reported on the influence of freezing and salting on meat proteins. Freezing for three, five, and ten days did not change the nitrogen distribution in fresh lean beef. Salting caused about ten per cent dehydration and about one per cent loss in nitrogen content, while enzyme processes were accelerated. Dry salting had only slight effect on water soluble nitrogen but retarded enzyme processes.

Several experiments relating to freshness of meat and its determination have been reported recently. Drosdov and Drosdov (1936) recorded changes in ammonia, volatile acid, glucose, pH, acid-alkaline balance, oxidation-reduction potential, and indol in beef stored at 3 to 5°C.(37.4 to 41°F.) and at 15 to 17°C.(59 to 62.6°F.). They stated that early indications of decomposition were rise in pH to 6.2-6.3 and production of 20 milligrams per cent ammonia, but in arriving at the state of decomposition all of the above factors were to be taken into consideration.

Lücke and Geidel (1935) determined volatile basic nitrogen, amino nitrogen (Van Slyke), acidity, and total nitrogen of fresh fish before and after different periods of storage. It was concluded that volatile basic-nitrogen determination was best for characterizing freshness of fish. Material which gave values of less than 30 milligrams per cent was considered edible. Analyses of chicken flesh made by Shoshin (1936) revealed no significant relationship between ammonia and hydrogen sulfide. He stated that presence of hydrogen sulfide did not prove spoilage, but ammonia content should be not over .025 per cent; the above value should be considered as a guide and not as an exact tolerance limit; bacterial count and the nature of the extractives should be taken into consideration.

Formation of indol in the decomposition of food cannot be used as an index of beginning of deterioration according to Smorodintsev and Diskina (1936). Indol was present only after decomposition had proceeded for some time. Electrical conductivity and freezing point of the extract of autolyzing beef muscle have been suggested as indices of freshness of meat by Smorodintsev and Kruilova (1935).

Use of ultra-violet lamps for aging beef has been reported by James (1936). The new process claims economies in time, shrinkage, and mold losses in aging or ripening of beef. In studying factors relating to color of meat Mackintosh and Hall (1936) found that

dark-colored beef changed to normal bright red color when it was ground and placed in an atmosphere of oxygen, also that the dark color appeared to result from some undetermined factor affecting the condition of the muscle hemoglobin rather than quantity of hemoglobin present. Lea (1935) reported that yellowing of bacon fat appeared to be a secondary reaction involving oxidation of the fat but the mechanism of the change as it occurred in the tissue remained undecided. In an investigation of the oxygen uptake of pork and bacon, as a factor in production and preservation of its color, Brooks (1936) found that the oxygen uptake of pork and beef muscles was about the same, while that of smoked bacon was lower. Absence of oxygen in the bulk of the tissue insured stability of color. In the presence of oxygen in bacon, the nitric oxide hemoglobin was changed to methemoglobin.

Inclusion of notes pertaining to bacteriology and food poisoning from meat and its products seem most appropriately presented in this section of the review. Tanner and Evans (1934), after studying the effect of meat-curing solutions on anaerobic bacteria, reported that up to .92 per cent sodium nitrite could not be relied on alone to prevent toxin formation by *Clostridium botulinum*, or spoilage by *Clostridium putrificum*, and *Clostridium sporogenes;* commercial curing mixtures were effective. Spoilage of cooked and uncooked meats was not prevented by 3.5 per cent salt in combination with .1 to 2 per cent sodium nitrate or .01 to .2 per cent sodium nitrite.

According to Jensen and Urbain (1936) the organisms responsible for green discoloration in meats were those that oxidized hemoglobin, nitrosohemoglobin, methemoglobin and hematin, and hydrogen sulfide forming bacteria. Spectroscopic characteristics of the compounds are presented; these compounds were different from methemoglobin, and it was suggested that they are probably oxidation products of hemoglobin.

Investigation of the cause of swells in commercially canned "roast" beef by McClung and Wheaton (1936) indicated that the anaerobic gas-producing bacillus *Clostridium welchii* was guilty; it was apparently not killed in the precooking procedure. It was believed not to constitute a public-health hazard as the organism and preformed toxin were destroyed in processing the canned product; it was also said that the toxin is not effective when administered orally.

Data on the lipolytic activities of many organisms on beef fat were recorded by Vickery (1936a and 1936b). This activity was found to be distributed in a random fashion among strains of each bacterial genus; Achromobacter, Pseudomonas, and Asporogenous yeasts were used in the tests.

Feldman (1936) prepared cultures of the left precural lymph node of 88 tuberculine swine carcasses and of the right internal iliac lymph node from 13 of them. Tubercle bacilli were obtained from four. This indicated, according to Feldman, that virulent tubercule bacilli may still be present in hog carcasses even though all visible lesions of tuberculosis have been removed.

According to Prescott and Geer (1936) refrigeration of properly prepared foods at temperatures below 10°C.(50°F.) will prevent formation of toxins by *Clostridium botulinum* for extended but undetermined periods. For the Salmonella group that temperature is not effective for security against bacterial growth; temperature maintenance below 3.9°C.(39°F.) is necessary for protection against these microörganisms.

In the food-poisoning literature relating to meat is a report by Kelly and Dack (1936) that for food-poisoning strains of Staphylococcus, broth, bread, and ham served as favorable media for production of an enterotoxic substance. Anderson, Phillips, and Tighe (1936), in some food-poisoning outbreaks in Cardiff and Swansea, suggested that the infected meat was contaminated with pigs' intestines during preparation. *Bacillus aertrycke* was isolated from the patient and also from faeces of two out of 57 pigs. Statistics on food poisoning by meat for recent years were recorded by Meyer (1935, 1936).

McNaught and Anderson (1936) concluded that since there are no practical methods of inspection for trichinous meat, the consumer must assume the responsibility of preventing trichinosis by thoroughly cooking fresh pork. Among 200 human diaphragms obtained at autopsy in San Francisco, 48 (24 per cent) were infected with *Trichinella spiralis.*

BIOCHEMICAL AND NUTRITIONAL COMPOSITION OF MEAT AND COMPARISON OF PROTEIN FOODS

H. E. Robinson

Numerous investigators have studied the composition of various meats. Sharpenak and Eremin (1935) reported methionine contents of various edible proteins. In beef, methionine was listed at 1.06 per cent of the total albumin or .21 per cent of the total meat. Cystine was found to be .23 per cent of the meat. Balboni (1934) has listed analytical tables for the flesh and viscera of the ox, calf, horse, pig, and lamb. The phosphorus contents of fresh and stored beef and pork as inorganic, acid soluble, and phosphogenic are given by Nagy (1935). Commercial beef contained 88.5 mg. inorganic, 237 mg. acid

soluble, 96.2 gm. phosphogenic, and 414 mg. of total phosphorus per 100 gm. The corresponding figures for pork were 86.8, 242, 98.8, and 425 mg., respectively. The analytical evaluation of desiccated pig's stomach was listed by Bullock (1936). Clinical activity of the samples analyzed was also noted. The chemical composition of fresh pig's stomach and various other visceral organs was determined by Smorodintsev and Palmin (1935). The calcium and magnesium content of the flesh of different animals was shown by Takamatsu (1936).

Watson (1935) has estimated the hemoglobin content of muscle. The hemoglobin was extracted with phosphate buffer and determined by conversion to acid hematin. The presence of acetone in normal beef muscle was found by Houdiniere (1935). The normal amount was 12 mg. per 100 gm. of tissue, while in a heifer with milk fever 23 mg. were found. Phosphocholine was isolated from beef liver by Inukai and Nakahara (1935). From 200 kilograms of beef liver there was obtained .3 gram of phosphocholine.

Nineteen samples of sausage and sausage meat were analyzed by Tobey (1936). A study of ham composition with special reference to the type of hog was made by Hankins and Ellis (1935).

Gravimetric methods for determination of total body protein and organ protein have been proposed by Addis, Poo, Lew, and Yuen (1936).

Certain of the basic amino acids of mixed proteins of ox muscle were determined by Rees (1936). Arginine 6.18 per cent, histidine .52 per cent, and lysine 7.18 per cent were found by means of Vicker's modification of Kessel's procedure.

The question of protein storage in liver has been studied by Luck (1936). Mammalian liver gave four fractions: globulin II, euglobulin, pseudoglobulin, and albumin. All of the liver proteins were stated to participate equally in the function of storage.

Hoagland and Snider (1936) have investigated the nutritive value of protein in calf lungs, cow udders, and hog spleens. Beef muscle had the highest value as a source of protein for maintenance and growth, followed closely by calf lungs and hog spleens, whereas, cow udders had much the lowest value. Various supplementary relationships were also studied.

Metabolism studies during an abundant protein diet were reported by Kashevnik, Neifakh, and Kharit (1935). Feeding experiments lasting more than a year were carried out with male metal workers and female textile workers. The men were given daily 225 to 250 grams of protein, the women 150 to 215 grams. All the usually accepted indices of nitrogen metabolism were the same as under the usual protein intake. A summary of clinical and laboratory findings on two

Arctic explorers who existed on an exclusive meat diet in a New York climate was given by Lieb (1935). The report covered examinations made seven years after the original experiments. In dietary studies on a vegetarian racing cyclist made by Wishart (1934) best results were obtained on high protein diets. Although the gross efficiency was lower than on diets poorer in protein, it was suggested that the small bulk and palatable form of protein-rich animal foods is an important factor.

Nuts were found to be an inadequate meat substitute by Mitchell and Beadles (1935). The protein biological values of beef, English walnuts, raw peanuts, roasted peanuts, and pecans were respectively 75, 56, 58, 56, and 75.

Stefansson (1936) offered a short discussion on blood as an antiscorbutic factor in diets almost exclusively of meat. The vitamin-A content of fresh beef, hog, calf, and lamb livers was determined colorimetrically by Holmes, Tripp, and Satterfield (1936). The Lovibond Blue units per gram for the livers were calf, 533; beef, 252; lamb, 235; and hog, 182.

The problem of the production of an undesirably soft carcass in farm animals owing to a diet of various nuts, oils, and soybeans has been under considerable study. Beard, Schulz, and Culbertson (1935) stated that experiments on steer calves indicated that the consumption of menhaden and coconut oils at the rate of 15 to 18.8 pounds per 100 pounds gain in weight was not injurious to health of the calf and had no deleterious effect on firmness of the carcass or on palatability of the beef. Sinclair (1936), in a study of the causes of soft bacon, fed pigs a basal ration supplemented with skim milk; it was found that variations in the proportion of oats and barley had little effect on firmness of the fat. Frost-damaged wheat did not produce softness. Changes in protein level of the diet could not be related to differences in fat constants. The condition of "softness" was related to thrift of the pig and to rate of growth. The possibility of breeding animals for fat firmness was suggested inasmuch as individuality appeared to be of importance. Roasted soybeans and cooked soybeans, in a study of the effect of soybeans, soybean oil meal, and tankage on the quality of pork reported by Vestal and Shrewsbury (1935), had high nutritive values and surpassed tankage, soybean oil meal, and raw soybeans in weight-gain production and reduction in feed requirements. Soft, low-melting fats were produced only when soybean products were fed in amounts exceeding 14 per cent of the ration. Soybeans should be fed as a supplement and not as a substitute for corn.

The influence of soybeans upon the gains, feed requirements, and character of the fat produced when fed to growing and fattening spring pigs on rape pasture was studied by Culbertson, Thomas, Beard, and Hammond (1936). They concluded that soybeans cannot be fed as more than five per cent of the ration for growing and fattening hogs without the production of undesirable carcasses. In a discussion of the soft-pork problem in Oklahoma Adams and Beall (1936) stated that soft pork cannot be avoided if softening feeds, such as peanuts, acorns, mast, and soybeans, are used exclusively to fatten market classes of hogs. Such feeds are not recommended for feeding hogs between 50 and 225 pounds.

Summer and winter rations for hog fattening were studied by Smith and Maynard (1935) who found that skim-milk powder produced larger gains but was not as economical as tankage. Equal parts of skim-milk powder and tankage produced higher gains in dry lot than tankage alone but were not so economical.

Manufacture of meat and bone meal and its use as a supplement for pigs was described by Plum and Simonsen (1935). The different classes of material and methods of preparation were discussed from the point of view of quality of the resulting product.

Ellis and Hankins (1935) measured the influence of protein content of the ration on the growth and fattening of hogs fed at moderately restricted levels. Physical and chemical analyses showed that hams containing the highest and lowest percentages of fat were produced by hogs receiving rations low in protein and medium in protein, respectively. Other carcass characteristics were not definitely affected by the protein content of the diet. No significant difference was noted in hog and rat tests of the nutritive value of packing-house by-products prepared by the wet and dry rendering process as reported by Shrewsbury and Vestal (1934).

Notwithstanding a disagreeable odor, the extract water from the manufacture of animal meal had no effect on the quality of the slaughter-house products according to de Ruyter de Wildt (1935). Bacon was solid and had a low iodine number.

When corn that had been treated with p-dichlorobenzene for weevil eradication was fed to hogs, Henley (1936) found that the meat was tainted with the odor and taste of the compound in less than two weeks' feeding time. After a four-week period on the treated corn, about 14 weeks on a p-dichlorobenzene-free ration were required for the meat to become edible.

The utilization of blood from abbatoirs for feeding purposes was investigated by Pfieffer (1935). Blood was dried at low temperature after absorption on ground cocoa shells and grain husks. In-

corporating such a product with other protein-rich materials gave good results in pig fattening and egg-laying trials.

A comprehensive series of tests on packing-house by-products in the diet of chickens on the production and hatchability of eggs has been concluded by Titus (1936). The by-products used in the tests included various kinds of meat meal, meat and bone meal, beef scrap, blood meal, and "stick." In general, egg production on diets containing such products was materially higher than on the basal unsupplemented diet. The experiments confirm previous evidence on the value of packing-house protein supplements in poultry rations. Harshaw (1936a) has studied the effect of diet, range, and fattening on the physical and chemical composition of cockerels. Several animal by-products, mineral supplements, fish oils, and plant products were used in the tests. Variations in diet seemed to have little effect on physical or chemical composition of the birds in the majority of cases.

The comparative value of soybeans and meat meal in poultry feeding was reported by Gutowska and Drescherowna (1936). The protein level used was balanced at 17 per cent. Growth was similar for six and one-half weeks, after which the group on meat meal was superior.

Rhodes, Bartel, and Jooste (1935) have been studying the effects of varying amounts of animal protein fed to pullets. The publication noted dealt with factors correlated with egg size and number of eggs. Pullets receiving either low, medium, or high protein rations showed positive correlations between maximum body weight and average annual egg weight.

Factors affecting the composition of market poultry were discussed by Harshaw (1936b). In these experiments a two-weeks' fattening period resulted in much greater change in the composition of the poultry carcass than did any of the variations in diet or range conditions.

Some causes and effects of a high, free, fatty-acid content of the meat scraps in poultry rations were considered by Schroeder, Redding, and Huber (1936). The use of meat scraps high in free fatty acid, over 30 per cent of total fat, increased the death rate and decreased the rate of growth and feed consumption in chicks. The poor results were attributed to an inactivation of vitamin A and not to the free fatty acids as such.

COOKERY

B. B. McLean

The work of Smith (1936) showed the specific effect of the phosphate ions in accelerating the change from gelatin to collagen at a

given temperature. In stewing meat the cooking time required to reach the desired degree of tenderness was lessened by the addition of monohydrogen and dihydrogen phosphates to give a pH between 6 and 7.

Although, according to Kaplan and Korpf (1936), antimony in enamel containers used for storage of lemonade has been responsible for cases of food poisoning, no cases have been traceable to cooking vessels. Until further work has been done, they advise against storing or cooking acid food in enamel utensils containing antimony. Enamel-lined cans for use in the canned-meat industry were described by Hallman (1936). The addition of a zinc compound as zinc oxide in resinous enamels to prevent sulfide discoloration in canned meats was stated to be a standard practice in the industry.

Monier-Williams (1935), in reviewing the occurrence and the behavior of aluminum in relation to food, concluded that aluminum should be excluded from food products but that there was no evident harm caused by the use of aluminum cooking utensils.

Increase in the calcium yield of breast of veal and of spareribs when cooked in acid solution of .033 normal was reported by Williams (1936). Under similar conditions the muscle tissue freed from bone cartilage and periosteum showed no increase in calcium. Increasing the acid content of cooking solution showed no increase in yield of calcium.

Various tests for the eating quality of meat have been devised. The paired-eating method for testing tenderness of meat was reported by Cover (1936). From statistical analyses there was a significant difference between two methods of cooking paired cuts of meat when judged by this subjective test.

Child and Fogarty (1935) described the use of the pressometer for determining quality and quantity of press fluid on meat samples. The report contained their observations on the effect of interior temperature upon semitendinous muscle of beef. The ratio of press fluids to dry matter was greater on muscle heated to 14.4°C.(58°F.) than that at 73.9°C.(75°F.). An inverse relationship was found between the total nitrogen content of press fluids and the interior temperature. Although there was an inverse relationship between percentage of press fluids and total cooking losses in muscles heated to 75°F., no relationship was indicated in muscle heated to 58°F.

A detailed account of methods and practices for comparative cooking tests for roasting meat was issued in bulletin form by Alexander and Clark (1934). The report deals with the influence of carcass grade, period of ripening, and method of cooking on shrinkage and rate of heat penetration during roasting of lamb and mutton legs

of various grades. The higher the grade the greater was the cooking loss owing to rendering of the fat. Increasing the length of the ripening period decreased the cooking time and shrink. From the results of the seven cooking temperatures used, it was concluded that lower cooking temperatures give less shrink. The stage of doneness made greater difference than did oven temperature on shrinkage.

Methods of canning and cooking meats with illustrations and recipes were compiled in bulletin form by Latzke (1936). The use of the meat thermometer in roasting meat gives the only accurate test for doneness. Low oven temperatures produce a roast more evenly cooked, with less shrinkage, and with greater juiciness. Recommended roasting temperatures are 135 to 162.8°C.(275 to 325°F.). Cooking losses were determined for various grades of dressed poultry by Maw, *et al.* (1936). Results showed a larger amount of edible meat and less total cooking loss in high-grade poultry. Because of a smaller amount of fat in "C" grade, the moisture loss was greater in it.

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